P. Thangavel · G. Sridevi *Editors*

Environmental Sustainability

Role of Green Technologies

Springer

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Foreword

The implementation of the principles of sustainability is a world's challenge in managing a life-sustaining and environmentally sound global ecosystem. Sustainability has become the foundation for developing modern environmental management strategies for safely consuming and protecting natural resources that meet needs of today's and future generations.

In comparing with conventional agriculture, sustainable agricultural systems include many farming practices that can both maintain crop production and improve soil environment health, such as uses of organic fertilizers, no-till or minimum tillage, polyculture, and biological pest management. Agriculture sustainability considers the utilization of more renewable energies including solar, wind, and biofuels and thereby reduces our dependence on non-sustainable energies (i.e., fossil fuel), inorganic fertilizers, and pesticides or herbicides. Importantly, sustainable agriculture also contributes significantly to global environmental conservation by the reduction of greenhouse gas emission, as well as evaluates carbon sequestration in agricultural soils. Indeed, exploring potential effects of sustainable farming practices on crop production and environmental protection will help us better understand the development and management of long-term agricultural sustainability, which also provides insight into developing other sustainable green technologies.

It has been well demonstrated that reducing agriculture's carbon footprint in sustainable agricultural production systems can also be effectively achieved from within the framework of *green technology*. Green plants and associated microbes can be used for environmental cleanup, a biotechnology called *phytoremediation*. This green technology includes several remediation processes, such as *phytoextraction*, *phytovolatilization*, *phytostabilization*, and phytodegredation. Phytoremediation technologies have been successfully applied for cleanup of inorganic- and organic-contaminated water and soils. Plant-based remediation processes have been well defined for many environmentally important contaminants, including heavy metals, metalloids, macronutrients, and persistent organic compounds (POPs). However, great effort is still needed to develop an effective phytomanagement system based upon the principles of agricultural sustainability. Using plants or trees successfully for field phytomanagement will be a long-term endeavor that requires multidisciplinary knowledge regarding plant selection, crop management practices, contaminant properties, and soil environmental conditions. Importantly, the production of viable plant products and the development of economically feasible remediation systems will encourage more widespread implementation of an integrated phytomanagement strategy. In this regard, oleaginous plants (*Brassica*) planted at phytoremediation field sites produced seed that has been successfully used to produce biodiesel fuels, while the residual seed meal can be used for animal feed or soil organic amendment.

To assess the appropriateness of sustainable environmental management strategies, suitable environmental indicators need to be developed and are applied for long-term monitoring with the determination of ecological functions, such as biological productivity, diversity, autonomy, and resilience, just to name a few. In regard to green or plant-based sustainable technologies, it is important to elucidate and better understand those limiting processes or parameters that are critical for effective management and long-term sustainability. To this end, more worldwide research will be needed for actively developing and implementing novel sustainable technologies and strategies in agricultural production and environmental management, such as biofuels and green economy. At present time, research continues in developing genetic engineering technology and its application that could result in economically efficient and environmentally sustainable biotechnologies, such as hyperaccumulating metals or nutrients or increasing plant's resistance to pests or chemicals. However, genetically modified plants will be subjected to special environmental regulations if they are applied for public or commercial remediation for the sake of protecting human and environmental health. Needless to say, a global effort will be needed to conduct research in different disciplines for developing long-term sustainability of the global ecosystem.

The different chapters contributed by experienced specialists provide a unique compilation of the dispersed literature on each topic. By sure, the readers of the book will benefit from this joint vision of different green technologies which is currently deployed for sustainable environmental management. Therefore, we believe that this book targets a potentially broad spectrum of audience at different hierarchical levels ranging from the graduate students/researchers to policy makers in this field of increasing importance.

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Preface

Sustainable environment is a paradigm for the future in which the four dimensions such as environment, society, culture, and economy are balanced to improve the quality of life. According to the Brundtland Report, sustainable development means the development that meets the needs of the present without compromising the ability of future generations. At the end of 2012, there were about 7.06 billion people in the world (US Census Bureau 2013) and it is expected to be more than 10 billion by 2100 (UN 2011). As a result, there is a need for clean water, food, and environment for all of them, and it is difficult to take care of everyone with depleted soil and chemical-laden drinking water. The only solution will be green technology, an eco-friendly technology which will conserve natural resources and ecosystems. According to the UNDP report in 2012, over 30 % of the food production goes waste every year (Gustavsson et al. 2011), but 40 % of the children in Africa who are below 5 years are malnourished (UNDP 2012). In the United Nations Conference on Sustainable Development, the "Zero Hunger Challenge" was launched by the UN Secretary General Ban Ki-Moon where all the countries will work for the future in which every individual would have adequate nutrition (UNCSD 2012). Sustainable consumption is a better way to reduce the resource use, degradation, and pollution and increase the quality of human life. The organizations like UNEP, WHO, and others focus on food waste reduction and launched the global campaign, "Think.Eat.Save: Reduce Your Foodprint," the theme of World Environment Day 2013. In addition, the World Food Day 2013 also focuses on sustainable food systems for food security and nutrition.

Renewable energy could account for 77 % of total primary energy supply by 2050. The past few years have seen a rise in green innovation, and increasing amounts of venture capital are flowing in, with India being rated as the third most attractive country for renewable energy investment. Green building concept have attracted both the building promoters and end users in terms of the cost-effective as well as healthy and comfortable living conditions such as minimum utilization of energy and water, conservation of natural resources and generates less wastes. According to UNEP (2010), green economy encompasses all the economic opportunities arising from actions that promote sustainability, improving "human well-being and social equity, while significantly reducing environmental risks and ecological scarcities." On the other hand, the contribution of environmental technologies to growing economy is known as "green growth" (OECD 2011). The green economy is expanding in the European Union and at the global level through clean technologies with green energy produced especially for wind turbines and biofuels. In addition, the green economy is also used in agricultural sectors such as different types of plant and animal breeds with high genetic performances, bioconversion of plant biomass, and green products obtained from bioreactors. The agricultural wastes and its by-products are mainly used in the production of heat and power, animal feed, or biogas by anaerobic digestion. Further, it is known that these materials may also contain high-value compounds such as antioxidants, pigments, and other molecules of interest. For example, quercetin extracted from onion waste is a potent antioxidant that has a positive effect against cancer (Murakami et al. 2008) and cardiovascular (Cook and Samman 1996) and neurodegenerative diseases (Ono et al. 2006).

Recently, most of the research on phyto-/bioremediation aspects have mainly focused on remediation of contaminated environments at different levels without affecting soil beneficial flora and fauna. Sustainable agricultural practices such as vermitechniques, biofertilizers, biopesticides, role of plant growth-promoting bacteria, and AM fungal in phytoremediation will also enhance the soil quality or soil health status. Suitable hyperaccumulator plants have also been used for dual benefit purposes such as phytoextraction and biofortification to solve the nutrient deficiencies especially in staple food crops. The UN's fourth World Water Development Report recommended broader collaborative and integrative water management approaches to avoid future conflicts over water among nations and, within nations, among farmers, urbanites, energy producers, environmentalists, and industries.

Green technologies mainly focus on renewable energy sources, sustainable agricultural practices, phyto-/bioremediation of contaminants, biofuels, sustainable utilization of resources, green buildings, green chemistry, and green economy. All of these eco-friendly technologies will help to reduce the amount of waste and pollution and enhance the nation's economic growth in a sustainable manner. We hope this book will bring out the recent advancement and application of different green technologies and strategies implemented worldwide and this will pave the way for sustainable environment. The contents of the book is aimed to provide an integrated approach to sustainable environment, and it will be of interest not only to environmentalists but also to agriculturists and forest and soil scientists and in bridging the gap between the scholars/scientists and policymakers.

We personally thank all the contributors of this book who have spent their valuable time and shared knowledge and enthusiasm. We express our sincere thanks to all our well-wishers, teachers, research students, and family. Without their unending support, motivation, and encouragement, the present grueling task would have never been accomplished. Exceptional kind support provided by Dr. Mamta Kapila, Raman Shukla, and their team at Springer deserves praises which made our efforts successful.

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Contents

Part I Sustainable Agriculture

Insight into the Role of Arbuscular Mycorrhizal Fungi in Sustainable Agriculture 3
P. Priyadharsini and T. Muthukumar

Recycled Water Irrigation in Australia 39
Balaji Seshadri, Nanthi S. Bolan, Anitha Kunhikrishnan, Saikat Chowdhury, Ramya Thangarajan, and Thammared Chuasavathi

A Review of Biopesticides and Their Mode of Action Against Insect Pests 49
Sengottayan Senthil-Nathan

Seaweeds: A Promising Source for Sustainable Development 65
T. Nedumaran and D. Arulbalachandran

Part II Renewable Energy

A Comprehensive Overview of Renewable Energy Status in India 91
Atul Sharma, Jaya Srivastava, and Anil Kumar

The Sahara Solar Breeder (SSB) Project Contributes to Global Sustainable Energy Production and Resource Conservation: An Overview 107
A. Boudghene Stambouli and H. Koinuma

Clean Development Mechanism: A Key to Sustainable Development 121
Himanshu Nautiyal and Varun

Microalgae as an Attractive Source for Biofuel Production 129
S. Ramaraj, S. Hemaiswarya, Rathinam Raja, V. Ganesan, C. Anbazhagan, Isabel S. Carvalho, and Niran Juntawong

Advancement and Challenges in Harvesting Techniques for Recovery of Microalgae Biomass 159
Amrita Difusa, K. Mohanty, and V.V. Goud

Part III Remediation Technologies

Characterization of *Bacillus* Strains Producing Biosurfactants 173
Anna Płaza Grażyna, Magdalena Pacwa-Płociniczak,
Zofia Piotrowska-Seget, Robin Brigmon, and Ewa Król

Production of Biosurfactants Using Eco-friendly Microorganisms 185
Chibuzo Uzoigwe, Christopher J. Ennis,
and Pattanathu K.S.M. Rahman

Eco-Friendly Technologies for Heavy Metal Remediation: Pragmatic Approaches 205
Hemambika Balakrishnan and Rajeshkannan Velu

Phytoextraction of Trace Metals: Principles and Applications 217
Tiziana Centofanti

Integrated Management of Mine Waste Using Biogeotechnologies Focusing Thai Mines 229
M.N.V. Prasad and Woranan Nakbanpote

Constructed Wetland: An Ecotechnology for Wastewater Treatment and Conservation of Ganga Water Quality 251
U.N. Rai, A.K. Upadhyay, and N.K. Singh

Mycorrhizal Plants' Accelerated Revegetation on Coal Mine Overburden in the Dry Steppes of Kazakhstan 265
D.V. Veselkin, A.N. Kupriynanov, Ju. A. Manakov,
A.A. Betekhtina, and M.N.V. Prasad

Part IV Green Economy and Green Nanotechnology

Drivers of Green Economy: An Indian Perspective 283
Sanjay Kumar Kar, Saroj Kumar Mishra, and Rohit Bansal

Green Nanotechnology: The Solution to Sustainable Development of Environment 311
Rajeshwari Sivaraj, Hasna Abdul Salam,
P. Rajiv, and Venckatesh Rajendran

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Part I

Sustainable Agriculture

Insight into the Role of Arbuscular Mycorrhizal Fungi in Sustainable Agriculture

P. Priyadharsini and T. Muthukumar

Abstract

Sustainable agriculture plays a vital role in agroecosystems and reduces adverse effects on the environment by utilizing the various natural processes. Optimum soil fertility is an essential goal to be achieved in sustainable agriculture system. The presence of beneficial microorganisms in the rhizospheric region and their activities are the main focal point which makes dynamic resources available to plants and conserve soil fertility. Majority of the agricultural and horticultural crops are associated with common soil fungi, the arbuscular mycorrhizal (AM) fungi. These fungi are crucial for plant health and fitness as they increase the efficiencies of the plant root systems. The hyphae of these fungi originating from roots grow into the soil and absorb nutrients especially phosphorus and deliver it to the roots. They also play a crucial role in imparting tolerance to plants against various stresses as well as modifying soil structure. Nevertheless, several agricultural practices involved in crop production can influence both AM formation and function. Consequently, AM fungal introductions or changes in crop management practices that enhance the proliferation, diversity and function of native AM fungi become essential. Optimization of agronomic practices that sustain maximum AM fungal presence and activity would enable to achieve increased plant production in sustainable agriculture.

Keywords

Sustainable agriculture • Arbuscular mycorrhizal fungi • Nutrients • Stress tolerance • Inoculum production

1 Introduction

In spite of the universal escalation of agriculture and tremendous growth in the major crop productivity over the last decades, the eradication of

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hunger facing mankind is far from being realized (FAO 2004). Factors that deter maximum crop productivity include several abiotic and biotic stresses like unfavourable climate, drought, diseases and pests. Recently, increased crop productivity has been achieved through crop breeding along with huge input of chemicals in the form of fertilizers and biocides. These chemicals not only disturb agricultural ecosystems but are also detrimental to the environment (Chapin et al. 2000; Barabasz et al. 2002; Parmesan and Yohe 2003; Zhong and Cai 2007). The tolerance of crops to various abiotic and biotic stresses can also be evolved through the exploitation of the worldwide abundant endophytic associations, where microorganisms live in reciprocally beneficial relationship with plants.

Sustainable agriculture, by definition, is ecologically sound, economically viable, and socially responsible (Siddiqui and Pichtel 2008). Agroecosystems are characterized by major dependence on human interference and therefore are influenced by factors that extend into the system such as energy, agrochemicals and their residues (Odum 1984). In contrast to natural ecosystems, agroecosystems are created and controlled by humans through the management of ecological processes for production and conservation. Soil is the prime area for manipulation in agroecosystems, because it is a biologically dynamic resource. Within this soil, the rhizosphere is the locus of greater role of energy flow and mineral cycling among the physical, chemical and biological components; it can therefore be considered as a subsystem (Wright and Miller 1994).

Arbuscular mycorrhizal (AM) fungi are one of the imperative soil microorganisms that participate mainly in the plant uptake of nutrients, especially phosphorus (P) in diverse agroecosystems (Atkinson et al. 2002; Gadd 2005; Jansa et al. 2008). In addition, AM fungi can easily take up and translocate other macronutrients and several micronutrients to plants (Ortas and Akpinar 2006; Abo-Rekab et al. 2010). Hence, AM fungi are recipients of worldwide attention as they play an important role in sustaining an active and diverse biological community essential for increasing the sustainability of agricultural systems (Gianinazzi and Schüepp 1994). Arbuscular mycorrhizal fungi constitute around 50 % of soil microbial biomass in agricultural soils due to their profuse growth and abundance (Olsson et al. 1999). Most of the major crops are capable of forming AM associations naturally and are the most common mycorrhizal type involved in agricultural systems (Barea et al. 1993). As AM fungal association can improve plant growth and health, there is an increasing interest in ascertaining their effectiveness in plant production systems and, consequently, in manipulating them when feasible, so that they could be successfully incorporated into plant production systems.

The aim of this review is to discuss the developments and to provide insights regarding the potentials of AM fungi in agricultural systems. Given the overview of beneficial effects of AM association on plant growth and health, it is expected that the development of appropriate management practices that enable the proliferation of AM fungi would reduce the chemical inputs (fertilizers and biocides) in the upcoming years, a key aspect of sustainable agriculture.

2 General Aspects

The obligate endosymbiont, ‘AM fungi’ associating with more than 90 % of terrestrial plants (Graham 2008), belongs to the phylum Glomeromycota and acts as a bridge between soil and plants. Arbuscular mycorrhizal fungal hyphae are coenocytic and aseptate and reproduce asexually by spores (Kuhn et al. 2001). Formerly called ‘vesicular–arbuscular mycorrhiza’ or ‘VAM’, the name implies to the production of special structures, i.e. arbuscules and vesicles (Fig. 1), within the host roots. However, the lack of the production of vesicles within the host roots by certain genera belonging to the order Gigasporales (*Gigaspora*, *Scutellospora*) resulted in the modification of term to ‘arbuscular mycorrhiza’ or ‘AM’. At present, there are around 249 species in 17 genera of fungi involved in AM association (Schüßler and Walker 2010).

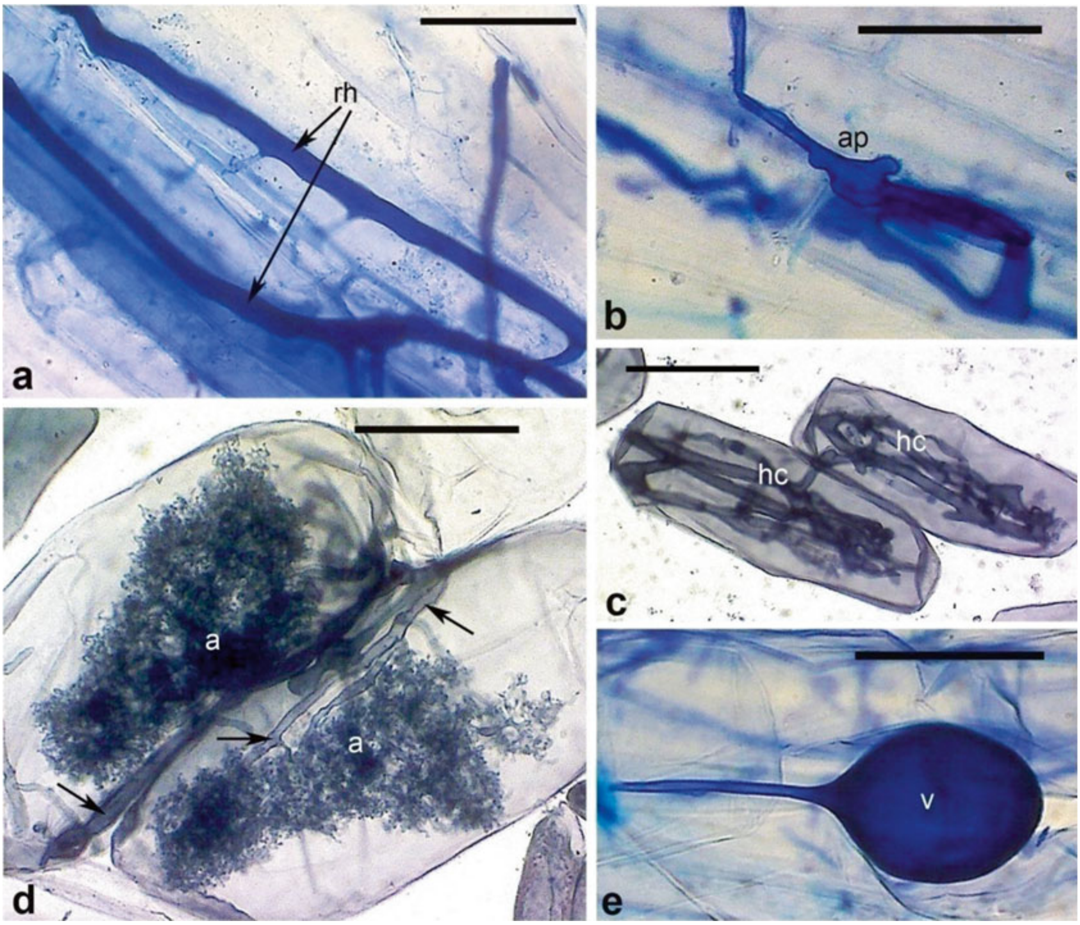

Fig. 1 (**a**–**e**) Arbuscular mycorrhizal colonization in crop plants. (**a**) Surface runner hyphae (rh) on root of *Allium cepa*. (**b**) Appressorium (ap) and hyphal entry in *Zea mays*. (**c**) Intracellular hyphal coils (hc) in *Capsicum annuum*. (**d**) Arbuscules (*a*) and arbuscular trunks (*black arrows*) in cortical cells of *A. cepa*. **e** Vesicle (*v*) in root of *Z. mays*. Scale bars = 50 µm

Like many host–microbe interactions, the colonization process begins with an exchange of signals between the two partners (host and the fungus), followed by the development of the symbiosis. The association is characterized by the adhesion and ingress of the fungus towards the host tissue. The host plant provides carbon source, the photosynthates to the fungus, whereas in turn, the extraradical hyphae of the fungus make available the soil nutrients that are not assessable to plant roots or to the host plant (Smith and Read 2008). The colonization of the root by an AM fungus begins with the fixation of the runner hyphae on the rhizoplane of a susceptible host through an appressorium (Fig. 1a, b). The AM fungal mycelium has dual phase: extraradical phase characterized by soil hyphae and intraradical phase characterized by exchange structures. The former is distinguished morphologically into two types: The first type is the runner hyphae (Fig. 1a) that actively transport nutrients and spread the hyphal network across the rhizospheric region extending the association to nearby plants (Smith and Read 2008; Neumann and George 2010). The second type is the finely branched fungal hyphae that play an important role in the uptake of nutrients from the soil. Intraradical phase consisting of intraradical hyphae, arbuscules and vesicles (Fig. 1c–e) plays an important role in nutrient exchange and uptake

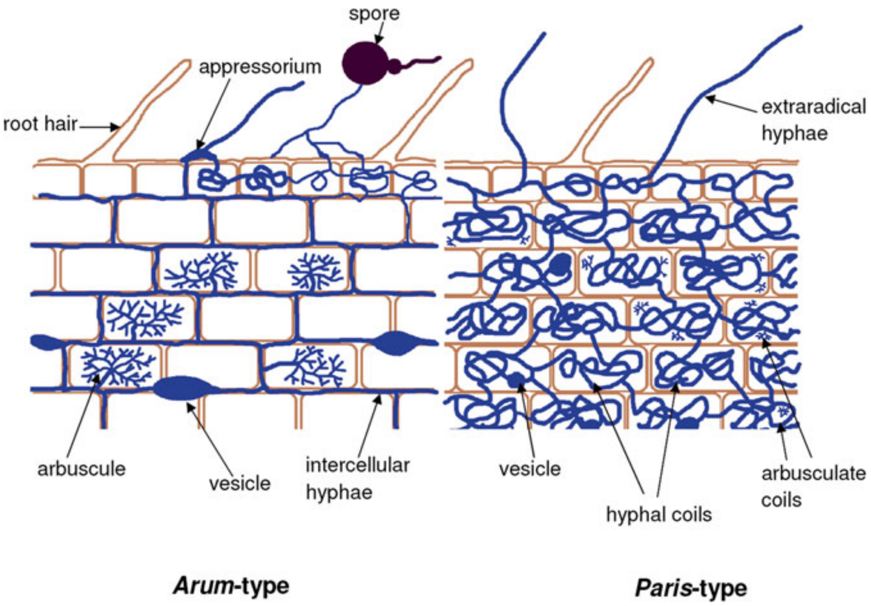

Fig. 2 Arbuscular mycorrhizal fungal structures showing *Arum*- and *Paris*-types

of carbon by the fungus. First, the AM hyphae receive suitable signals from host roots in the form of root exudates, and most specifically strigolactones (Akiyama et al. 2005; López-Ráez et al. 2008), which results in the branching of the hyphae. In response, the branched hyphae secrete a diffusible signal to the host roots, which initiates the expression of symbiotic-related genes (Kosuta et al. 2003). Based on the distribution of AM fungal structures within the roots, AM colonization patterns within host roots are divided into three types: *Arum*-, *Paris*- and intermediate-types (Dickson 2004).

In the *Arum*-type, the hyphae grow intercellularly in the root cortex and penetrate to form 'arbuscules' intracellularly, whereas in *Paris*-type association, intracellular hyphal coils frequently having intercalary arbuscules spread cell to cell in the cortex (Fig. 2). Intermediate-type AM exhibits characteristics of both *Arum*- and *Paris*-types. Most of the cultivated crops form *Arum* type, while *Paris*-type is common in plants of natural ecosystem (Ahulu et al. 2005).

Though most agricultural crops such as flax (*Linum usitatissimum*), corn (*Zea mays*), rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), potato (*Solanum tuberosum*), sugarcane (*Saccharum officinarum*), tomato (*Lycopersicon esculentum*) and sunflower (*Helianthus annuus*) can benefit from mycorrhizal association, certain crops belonging to Amaranthaceae, Brassicaceae and Chenopodiaceae do not form AM symbiosis (Brundrett 2009).

A wide range of AM fungi have been found to be associated with crop species (Fig. 3). In spite of the general assumption that the diversity of AM fungi is low in agricultural soils, several studies have reported high AM fungal diversity in agricultural soils (Jansa et al. 2002; Oehl et al. 2003, 2004; Ambili et al. 2012). Many studies on the diversity of AM fungi in agricultural soils have indicated the dominance of AM fungal communities by species belonging to the genus *Glomus* or the species that were once under *Glomus* (Jansa et al. 2002; Muthukumar and Udaiyan 2002; Sjoberg et al. 2004; Mathimaran et al. 2005). Nevertheless, spores of AM fungi belonging to *Acaulospora*, *Entrophospora*, *Gigaspora*, *Sclerocystis* and *Scutellospora* have also been reported along with *Glomus* in agricultural soils (Jansa et al. 2002; Muthukumar and Udaiyan 2002).

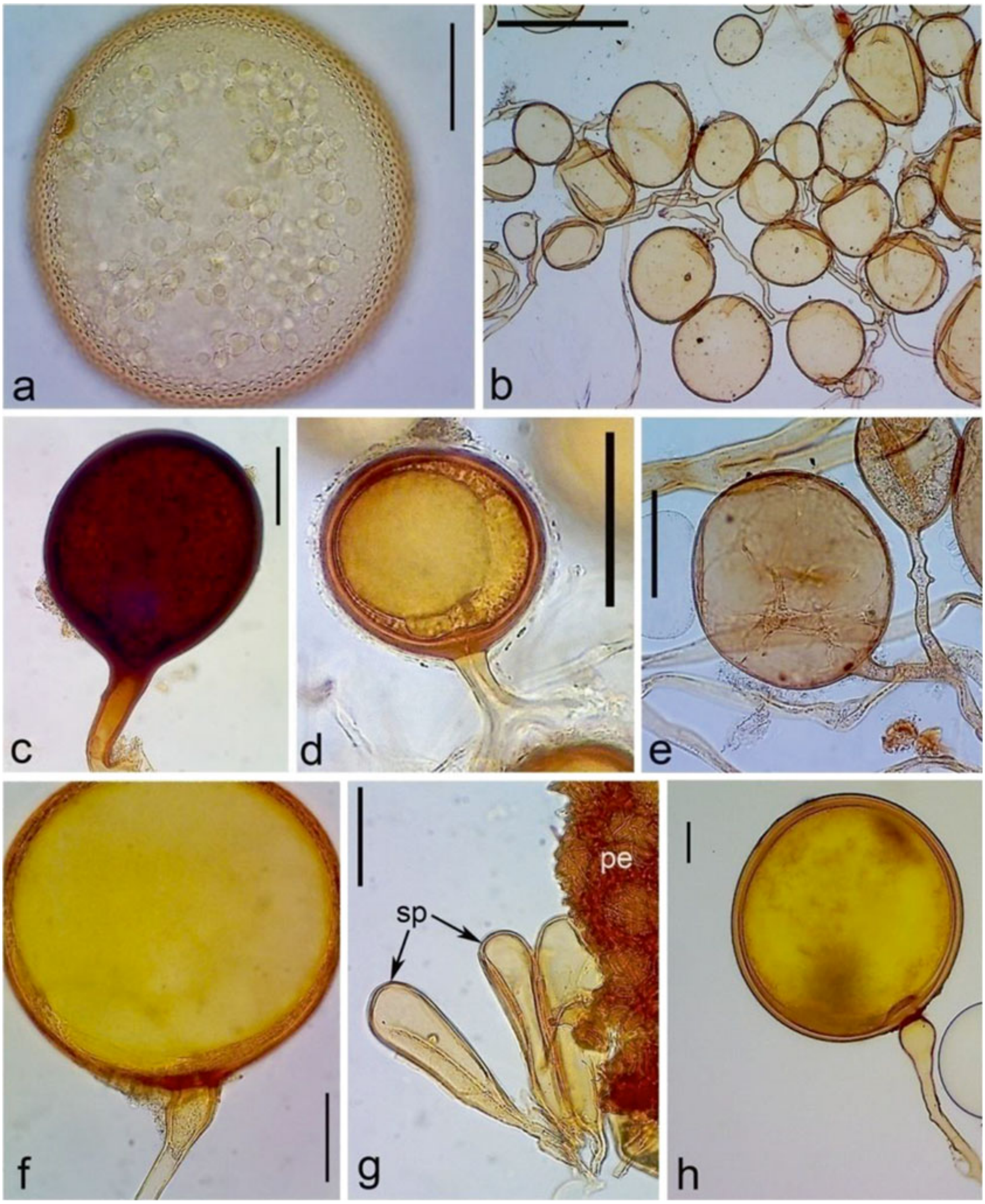

Fig. 3 (**a–h**) Spores of arbuscular mycorrhizal fungi associated with *Eleusine coracana*. (**a**) Spore of *Acaulospora scrobiculata*. (**b**) Loose cluster of *Glomus aggregatum*. (**c**) *Funneliformis geosporum*. (**d**) *Rhizophagus fasciculatus*. (**e**) *Rhizophagus intraradices*. (**f**) *Funneliformis mosseae*. (**g**) *Sclerocystis sinuosa* (*pe* peridium, *sp* spore). (**h**) *Scutellospora calospora*. Scale bars = 50 μm

3 Effects of AM Fungi

The major effects of AM association on host plants include enhanced uptake of low-mobile ions, nutrient cycling, rooting and plant establishment, plant tolerance to various biotic and abiotic stresses, improved soil quality and structure and enhanced plant community diversity. In agricultural ecosystems, AM fungi play a vital role in maintaining sustainability (Sanders 2004), by enhancing crop growth (Meir et al. 2010) and productivity (Lekberg and Koide 2005), soil constituents and fertility (Piotrowski et al. 2004; Li et al. 2007) and pathogen resistance (Sikes et al. 2009).

3.1 Improved Nutrient Uptake and Nutrient Cycling

Arbuscular mycorrhizal fungi improve plant uptake of nutrients by increasing the plant surface area of absorption. The narrow diameter of the absorbing hyphae allows more nutrients to be taken up from the soil solution. Generally, nutrient depletion zones develop around the root when the nutrients are removed from the soil solution by the plant roots (Fig. 4). For poorly mobile ions such as phosphate, a sharp and narrow depletion zone develops very close to the root. Hyphae of AM fungi can readily spread beyond this depletion zone and take up additional phosphate from the soil (Li et al. 1991) (Fig. 4). The uptake of other nutrients like N, K and micronutrients is also improved by AM fungi because many of these elements are also limited due to various reasons in the soil.

Two important factors that contribute to the effective uptake of nutrients by AM fungi from the soil are (i) the narrow diameter of the fungal hyphae and (ii) its longer lifespan relative to root and root hairs. As the diffusion gradient for a nutrient is inversely related to the radius of the absorbing unit, the soil solution should be less depleted at the surface of a narrow absorbing unit such as hyphae. Further, narrow hyphae can also grow into small soil pores that are not accessible to roots and root hairs (O'Keefe and Sylvia 1991). Therefore, crop species with well-developed root systems with fine roots or abundant root hairs like wheat, barley and oats (*Avena sativa*) remains little affected by AM colonization (Ryan and Graham 2002).

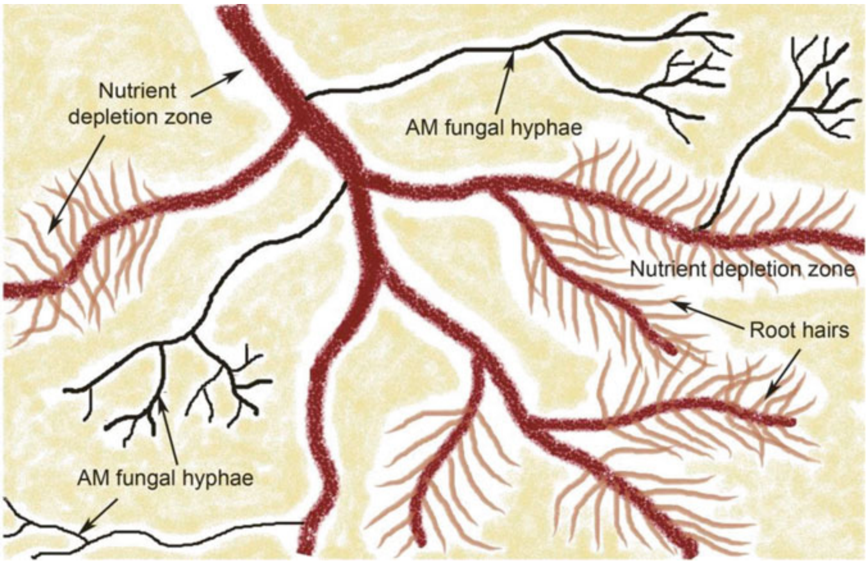

Fig. 4 Arbuscular mycorrhizal fungal hyphae and nutrient depletion zones around root

Arbuscular mycorrhizal fungi participate in N dynamics that relate in N cycling, plant growth and ecosystem functioning (Miransari 2011). The reduction of NO_3 is of environmentally significant concern. This has been accomplished with the presence of AM fungi (Hodge and Fitter 2010; Miransari and Mackenzie 2010), which absorb and transfer the N to the host under various conditions (Liu et al. 2007; Atul-Nayyar et al. 2009; Tian et al. 2010). Symbiotic N_2 fixation, the starting point in the N cycle, depends on an adequate and steady supply of P to the root and nodules (Barea et al. 1993). The AM fungi play an important role in enhancing growth, nodulation and N_2 fixation by legume crops symbiotic with nodulating bacteria. An increased N_2 fixation of mycorrhizal crop plants both under control (Kucey and Bonetti 1988; Barea et al. 1989a) and field conditions (Barea et al. 1989b; Shivaram et al. 1988) has been adequately demonstrated.

3.2 Plant Tolerance to Stresses

3.2.1 Abiotic Stresses

3.2.1.1 Water Relations

Water is an essential component for plant growth which is affected by global climatic change. Drought is one of the most important abiotic stresses that limit the crop growth and yield in agroecosystems in both arid and semiarid regions (Feng et al. 2002). The symbiotic association of plants with AM fungi has been shown to enhance plant tolerance to drought (Ruiz-Lozano et al. 2006; Boomsma and Vyn 2008). In arid regions, minimum moisture content in plants is balanced by an increased uptake of water by roots through AM fungal hyphae (Khan et al. 2003). Positive influence of AM fungi in improving plant water use efficiency and sustaining drought has been shown for wheat (Al-Karaki et al. 2004), oats (Khan et al. 2003), corn (Subramanian et al. 1997; Subramanian and Charest 1999), soybean (*Glycine max*) (Aliasgharzadeh et al. 2006), garden pea (*Pisum sativum*) (Quilambo et al. 2005), onion (*Allium cepa*) (Bolandnazar et al. 2007), tomato (Subramanian et al. 2006) and other crop species (see Augé 2001). It has been shown that an increased nutrient uptake mediated through AM fungi could impart more resistance to drought in mycorrhizal plants (Ruiz-Lozano et al. 1995). The increased uptake of P by AM plants under drought conditions results in higher yield than those without AM fungi (Smith and Read 2008). Therefore, improved P nutrition by AM fungi during the periods of water deficit has been postulated as a primary mechanism for enhancing host plant drought resistance under water stress conditions (Subramanian et al. 2006). In contrast, others consider that host plant drought tolerance is independent of P uptake stimulated by AM fungi (Davies et al. 1993; Augé et al. 1994). In addition to P, mycorrhizal plants can also absorb more N under drought conditions resulting in increased growth and yield (Tobar et al. 1994; Subramanian et al. 2006). One of the widely accepted mechanisms of AM symbiotic influence on plant water relation involves the AM fungal effect on plant size. The response of plants to mycorrhizal colonization is often related to the direct influence of AM fungus on plant size in conjunction with improved P nutrition (Ebel et al. 1994). However, mycorrhizal effect on metabolic changes (Subramanian and Charest 1995) and modified N assimilation pathways (Subramanian and Charest 1998) as shown earlier can also influence the size of host plants. The AM fungi could therefore to a certain extent replace genetic engineering and plant breeding techniques (Xu et al. 2008; Grover et al. 2011) by modifying the crop plant physiology as well as biochemical responses (Kohler et al. 2008; Grover et al. 2011) to stress tolerance. For example, AM fungal association has been shown to increase the stomatal conductance of mycorrhizal mutant bean (*Phaseolus vulgaris*) than non-mycorrhizal under water deficit condition (Augé 2004).

3.2.1.2 Salinity

Salinity is one of the cosmopolitan threats to crop production worldwide. Irrigation with groundwater and irrational use of easily soluble fertilizers are main causes for salinity in agroecosystems (Copeman et al. 1996; Al-Karaki 2000). It has been estimated that more than 250 million ha of groundwater irrigated lands is salinized, of which ten million ha is abandoned annually (Codevasf 2011). Salt deposition in the soil results in hyperionic and hyperosmotic stresses (Evelin et al. 2013). The presence of excess salts in the soil solution may limit the growth of an organism due to specific ion toxicity or osmotic stress. These factors tend to differ in relative importance depending on the species and concentration of ions involved, as well as the tolerance of the organism in question (Brownell and Schneider 1985). Salinity may affect certain stages of the life history of an organism more compared to other stages. Salt stress induced decline in crop productivity results from its negative impact on plant growth and development (Giri et al. 2003; Mathur et al. 2007). Arbuscular mycorrhizal fungi have been shown to increase crop yield under saline soils (Daei et al. 2009). Nevertheless, results on the influence of salinity on AM formation and function are often contradictory. Some studies have shown that soil salinity reduces root colonization by AM fungi and increases plant's mycorrhizal dependency (Tian et al. 2004; Sheng et al. 2008). In contrast, it has also been shown that AM colonization either remains unaffected (Yamato et al. 2008) or even increased under salt stress (Aliasgharzadeh et al. 2001). An increased soil salinity has also been shown to adversely affect the production of extraradical hyphae of AM fungal strains that are sensitive to salinity (Juniper and Abbott 2006; Evelin et al. 2009). The extent to which salinity reduces AM colonization depends on the stage of the association such that inhibition is more prominent during early stages of the symbiosis development than during the later stages (McMillen et al. 1998). For example, salinity inhibited early colonization of roots by *Gigaspora decipiens* more than by *Scutellospora calospora* (Juniper and Abbott 2006). It has been shown that AM fungi alleviate salt stress in some plants through modifications in physiological mechanisms (see Evelin et al. 2009; Porcel et al. 2012). However, the adjustment of osmotic potential by settling down of soluble sugars in mycorrhizal fungal parts has been suggested to protect the plant from salinity (Soliman et al. 2012). For instance, trehalose in spores and extraradical mycelium enables AM fungi to colonize host plants even under high salinity (Schubert et al. 1992). Several studies have reported that salt stress induces modifications in plants even at ultrastructure levels (Yamane et al. 2004; Miyake et al. 2006; Andrea and Tani 2009). Recently, Evelin et al. (2013) showed that the ultrastructural changes in AM-inoculated fenugreek (*Trigonella foenum-graecum*) plants exposed to four different levels of salt were less than non-mycorrhizal plants. Studies have also shown that some AM fungi are able to adapt to different environmental conditions better than others (Stahl and Christensen 1991). Thus, the varied observations reported by different workers may partly reflect the differences between the fungi used and their ability to adapt to various environments. Nevertheless, most of studies examining mycorrhiza and soil salinity to date have not considered these differences.

Arbuscular mycorrhizal fungal-mediated salt stress tolerance has been shown for crops like chilli (*Capsicum annuum*) (Çekiç et al. 2012), Chinese milk vetch (*Astragalus sinicus*) (Peng et al. 2011), pepper (*Piper nigrum*) (Turkmen et al. 2008; Kaya et al. 2009), fenugreek (Evelin et al. 2012), corn (Sheng et al. 2008, 2011), bajra (*Pennisetum glaucum*) (Borde et al. 2011), tomato (Hajiboland et al. 2010) and clover (*Trifolium alexandrinum*) (Gharineh et al. 2009). Like drought stress, an increased P uptake mediated by AM fungi has been suggested to alleviate saline stress (Tian et al. 2004). However, in certain cases, saline tolerance of mycorrhizal plants appears to be independent of P concentration (Feng et al. 2002). Both differences in the ability between AM fungi to obtain P from the soil and their ability to adapt to changing edaphic conditions (del Val et al. 1999) could reason for varied sensitivities of AM fungi to salinity. Therefore, it might be expected that an isolate originating from saline soil would have a higher adaptability and a greater capacity to promote plant growth under saline stress.

3.2.2 Biotic Stress

3.2.2.1 Protection Against Pests and Pathogens

In agriculture, pest and pathogen infestations severely damage the crops, resulting in a decline in crop yield. In spite of constant efforts to eradicate these pests and pathogens using chemical agents, little and temporary success has been achieved. Alternatively cost-effective biological methods involving microbes could be used to improve host plant resistance against pests and pathogens. There is a direct competition in host roots for nutrient uptake and proliferation between AM fungi and pathogens as they colonize the same niche. Some of the recent studies do provide evidence that AM fungi and their interaction with plants could substantially reduce the damage caused by soilborne pathogens (Whipps 2004; St-Arnaud and Vujanovic 2007; Smith and Read 2008). Further, the extent of protection imparted by AM fungi could vary with the pathogens and the host plant involved. Nevertheless, the degree of protection imparted by AM symbiosis against pests and pathogens could be modified by soil and other environmental conditions. Mechanisms by which AM fungi control root pathogens include (i) improved nutrient status of the host, (ii) damage compensation, (iii) competition for host photosynthates, (iv) competition for infection sites, (v) anatomical and morphological changes in the root system, (vi) microbial changes in the mycorrhizosphere and (vii) activation of plant defence mechanisms. In some cases, the direct biocontrol potential of AM has been demonstrated, especially for plant diseases involving pathogens like *Phytophthora*, *Rhizoctonia* and *Fusarium* (Abdel-Aziz et al. 1997; St-Arnaud et al. 1997; Vigo et al. 2000). A recent study by Singh et al. (2013) has clearly demonstrated the AM fungal ability to efficiently control *Fusarium* wilt disease under all conditions in three chickpea (*Cicer arietinum*) varieties tested. Further, several studies have also confirmed the existence of synergism between AM fungi and biocontrol agents such as *Burkholderia cepacia* (Ravnskov et al. 2002), *Pseudomonas fluorescens* (Edwards et al. 1998), *Trichoderma harzianum* (Datnoff et al. 1995) and *Verticillium chlamydosporium* (Rao et al. 1997). These interactions suggest that AM might affect plant and soil microbial activity by stimulating the production of root exudates, phytoalexins and phenolic compounds (Norman and Hooker 2000; Bais et al. 2005). A small increase in the activity of plant defence genes, especially those involved in the production of chitinases, glucanases, flavonoid biosynthesis and phytoalexins, has been observed during mycorrhizal growth; however, these mycorrhizal defence induction mechanisms remain transitory (Guillon et al. 2002; Harrier and Watson 2004). Further, AM-mediated resistance to biotic stress could vary with the mycobiont involved. For example, Ozgonen and Erkilic (2007) used three different species of Glomeraceae [*Funneliformis mosseae* (=*Glomus mosseae*), *Claroideoglomus etinicatum* (=*Glomus etunicatum*), *Rhizophagus fasciculatus* (=*Glomus fasciculatum*)] and a Gigasporaceae species (*Gigaspora margarita*) to control blight disease caused by *Phytophthora capsici* in pepper. The results of the study clearly showed a significantly higher plant growth and reduced disease severity in AM-inoculated plants. Of the different species of Glomeraceae screened, *F. mosseae* was found to be more efficient than others.

Many studies have also reported the suppressive effect of AM fungi on sedentary endoparasitic nematodes (Elsen et al. 2003; de la Peňa et al. 2006). In some crops, this effect is significant enough to the level, to consider AM fungi to be more or less an efficient means of biological control (Castillo et al. 2006). With migratory endoparasitic nematodes, studies have demonstrated a decrease in nematode population development like *Meloidogyne incognita* on cucumber (*Cucumis sativus*) (Zhang et al. 2008), *Radopholus similis* on banana (*Musa* spp.) (Elsen et al. 2004; Jefwa et al. 2010), *Pratylenchus* on dune grass (*Ammophila arenaria*) (de la Peña et al. 2006) and *Rhizoctonia solani* (Yao et al. 2002) on potato. Recently, Affokpon et al. (2011) evaluated native and commercial AM fungi for their efficacy to protect plants against root-knot nematode, *Meloidogyne* spp. The results of this study indicated that the

Table 1 Interaction of arbuscular mycorrhizal fungi with beneficial soil microorganisms

Group of microorganisms	Results
Symbiotic and asymbiotic N_2 fixers	N_2 fixation, N cycling, N transfer
Phosphate solubilizers	P cycling, use of sparingly soluble P source
Phytostimulators	Increased rooting and seedling establishment
Biocontrol agents	Increased resistance/ tolerance to root disease
Other fungi and bacteria related to soil aggregation	Important to soil quality

Adapted from Azcón-Aguilar and Barea (1997) with permission

nematode attack could mask the magnitude of AM fungal benefits to the host plant, but AM fungal isolates could modify the severity of stress on plants to different levels (Veresoglou and Rillig 2012).

3.2.2.2 Interaction with Other Soil Organisms

Arbuscular mycorrhizal fungi interact with a diverse group of organisms in the rhizosphere. These interactions can range from positive to neutral and to negative on the AM association or a particular component of the rhizosphere (Azcón-Aguilar and Barea 1992; Rillig 2004). Different types of positive interactions between AM fungi and other soil microorganisms are presented in Table 1. The microflora in the rhizosphere of mycorrhizal roots most aptly termed as the 'mycorrhizosphere' quantitatively and qualitatively differs from the non-mycorrhizal roots (Bansal and Mukerji 1994). Mainly two groups of bacteria, namely, saprophytes and symbionts, interact with AM fungi that may be either detrimental, neutral or beneficial in their response (Johanson et al. 2004). 'Mycorrhiza helper bacteria' (MHB) found mostly in temperate and tropical ecosystems (Frey-Klett et al. 2007) initiate AM fungal root colonization, stimulate mycelia growth and also assist spore germination (Gryndler et al. 2000; Vivas et al. 2006). The interaction between AM fungi and nodulating nitrogen fixers has received considerable attention because of the high P demand involved in N_2 fixation (Barea and Azcón-Aguilar 1984; Veresoglou and Rillig 2012). The two symbionts act synergistically under low fertile conditions, resulting in greater N and P content in dually inoculated plants than when the organisms are inoculated separately. Even at low water potential, AM fungal inoculation improves nodulation and N_2 fixation by the bacterial symbiont (Goicoechea et al. 1998), thereby neutralizing the effects of salinity. An early association of the seedling with AM fungi can moderate the stressed condition of the host (Evelin et al. 2009). It has been noted that the premature nodule senescence in soybean under drought conditions could be ameliorated through AM fungal inoculation (Porcel et al. 2003).

Synergistic interactions have also been reported between AM fungi and plant growth-promoting rhizobacteria (PGPR) (Muthukumar et al. 2001; Muthukumar and Udaiyan 2006; Sala et al. 2007). However, the nature and the extent of benefit from interaction could vary. For instance, Chandanie et al. (2005, 2006) noted that co-inoculation of *Trichoderma* with an AM fungus (*F. mosseae*) positively stimulated plant growth. However, no such effect on plant growth was evident when *Penicillium* was co-inoculated with the same AM fungus.

3.2.2.3 Nutrient Transfer in Intercropping Systems

Intercropping is an ancient technique of growing more than one crop species simultaneously in the same field. It plays an important role in agriculture rendering advantages to both soil and plant. Intercropping improves soil texture and soil water availability and supplies various organic matters for most efficient proliferation of symbiotic and non-symbiotic microorganisms (Burner 2003; Muok et al. 2009). The wide and diverse plants in an intercropping favour an increased and viable population of AM fungi. However, the capability of legumes to form dual symbiotic association with both bacteria and AM fungi is important in intercropping systems from improving soil fertility point of view (Pagano et al. 2008; de Carvalho et al. 2010). As plants from different species could be linked by the common AM mycelia network, intercropping of legume crops can benefit nonleguminous crops through the transfer of N via common mycelia network (CMN) (Simard and Durall 2004). This CMN aids nutrient transfer between different host plants, thereby acting as an extension of the root systems, and also provides signalling molecules (Xiaolin and Shang 1997). For example, the cocultivation of citrus (*Citrus tangerine*) which is highly dependent on AM fungal association due to the poor root development (Wu and Xia 2006), along with a leguminous herb, *Stylosanthes gracilis*, consequently increased both soil quality and citrus yield in Southern China (Yao et al. 2008).

Other features of intercropping like the nutrient cycling and organic matter turnover can maximize the resource use by plants, thereby improving soil fertility. For example, Li et al. (2007) showed that the root exudates of faba bean containing organic acids and protons increased P content of maize plants in a maize–faba bean (*Vicia faba*) intercropping system. The CMN of AM fungi also enhances P balance as well as the N and P levels between plants (Giovannetti et al. 2004). Each AM fungal species tends to have different effects in relation to plant systems. For instance, AM fungal taxa that reduce plant growth in one plant species can enhance it in another (Klironomos 2003). Recently, Hu et al. (2013) examined the effect of intercropping *Sedum alfredii* with *Ipomoea aquatica* inoculated with two different AM fungal species ([Funneliformis caledonium (=*Glomus caledonium*) 90036, *Glomus versiforme* HUN02B)] in cadmium (Cd)-contaminated soil. The AM fungus *F. caledonium* 90036 increased P acquisition and plant biomass of *S. alfredii*, whereas *G. versiforme* HUN02B had the same effect on *I. aquatica*. Some of the studies also suggest that intercropping is beneficial and far better than monoculture system (Harinikumar et al. 1990; Ishii et al. 1996).

3.2.2.4 Rooting

Nutrient supply by the AM mycelium activity exerts a feedback regulation, especially in the aerial parts of the plant like photosynthesis and the translocation of the photosynthates. Generally, fewer photosynthetic products are allocated to the root due to an increased efficiency of the roots in response to AM symbiosis (Smith et al. 2003; Gamalero et al. 2004); the shoot/root ratios of AM plants are usually higher in AM plants than in their corresponding non-AM controls (Smith 1980). It has also been recently recognized that AM colonization could affect a wide range of morphological parameters in developing root systems including root branching (Atkinson et al. 1994; Berm et al. 1995). Enhanced root proliferation in response to AM fungal inoculation has been reported in black pepper (*Piper nigrum*) (Anandaraj and Sarma 1994; Thanuja et al. 2002) and cashew (*Anacardium occidentale*) (Krishna et al. 1983). Therefore, it has been speculated that changes in the plant hormonal balance and meristematic activity in response to AM association were responsible for the AM-induced effects on root development.

Changes in root morphology and P uptake alter the rhizosphere through predominantly affecting the microbial community (Linderman 1988). The variation in the root architecture of mycorrhizal plants from that of a non-mycorrhizal plant clearly indicates the involvement of some compounds from root system responsible for these traits. For example, Wu et al. (2010) showed that polyamines in addition to improving plant growth could also significantly alter the root system architecture in AM plants.

4 Management of AM Fungi

In agroecosystems, various management practices such as the degree and type of fertilization, plant protection, fallow period and soil tillage could influence AM association.

4.1 Fertilizer

Crops require adequate nutrients especially P during early stages of growth for optimum crop production (Grant et al. 2001). Limited P supply frequently limits crop production and P fertilizer is commonly applied to ensure that sufficient P is available for optimal crop yield and maturity (Grant et al. 2005). The total soil P usually ranges from 100 to 2,000 mg P kg^{-1} soil representing approximately 350–7,000 kg P ha^{-1} in surface 25 cm of the soil, although only a small portion of this P is immediately available for crop uptake (Morel 2002). However, for AM fungi which are known for its P uptake (Hu et al. 2009), soil P is one of the major deterrent soil factors in agricultural systems that affects AM. It has been well proved that the AM fungal benefit tends to decline as the concentration of P in the plant increases (Valentine et al. 2001). Higher tissue P reduces the production of external hyphae (Bruce et al. 1994), hyphal branching (Nagahashi and Douds 2000) and sporulation (De Miranda and Harris 1994) of AM fungi. As available P in the soil increases, AM association may depress plant growth, as there is a carbon cost associated with supporting the association (Kahiluoto et al. 2000). For example, cucumber plants inoculated with AM fungi and raised on full-strength nutrient solution had 19 % lower biomass than uninoculated plants (Valentine et al. 2001). In contrast, mycorrhizal plants had 66 % higher biomass compared to non-mycorrhizal plants under reduced P concentration in the nutrient solutions. However, the effect of P fertilization on AM fungi may vary with P sources. While readily soluble or available, forms of P (inorganic) affect AM association to a greater extent than less soluble forms of P (e.g. rock phosphate) (Linderman and Davis 2004). Similar results have also been observed for N fertilization (Gryndler et al. 1990; Liu et al. 2000). The effect of P fertilization often changes with the response or balance of other nutrients present. The AM fungal benefit and mycorrhization tend to be highest when low P is combined with an ample supply of other nutrients (Grant et al. 2005). For instance, Guttay and Dandurand (1989) observed an increased mycorrhization in corn with N and K fertilization at low P levels but a decrease at high P levels. This clearly suggests the interactions among N, P and K fertilization in corn. The application of NPK fertilizer along with AM fungi has been shown to increase plant growth in potato (Eliopoulos et al. 2007), onion (Gergon et al. 2008) and cucumber (Ahmed et al. 2009). Using AM as a bioinoculant, instead of phosphate, was found to have a direct impact on sugarcane growth and yield (Surendran and Vani 2013). Like for AM colonization, some reliable evidence does indicate that the use of fertilizers can reduce AM fungal spore populations in the soil (Bhadalung et al. 2005; Emmanuel et al. 2010).

In organic farming, the use of synthetic fertilizers is avoided which enables the crops to depend on AM fungi for soil nutrients (Galvez et al. 2001). In addition, organic fertilization enhances AM fungal association and formation of AM fungal propagules in the soil (Gryndler et al. 2005; Gaur and Adholeya 2005), thereby improving soil quality. Though organic fertilizers generally have a positive effect on crops as evidenced by enhanced growth and accumulation of nutrients (Silva et al. 2007; Sharif et al. 2012), results of some studies are found to be opposite (Martin et al. 2002; Elorrieta et al. 2003). A consortium of seven AM fungi isolated from the soils of coffee (*Coffea arabica*) plantations with different fertilizer inputs (low, intermediate and high) was examined for their growth-promoting ability in coffee both under nursery and field conditions. The results of this study clearly showed that greater fertilizer inputs negatively influenced the spore abundance and plant growth, whereas intermediate input increased the AM fungal abundance (Trejo et al. 2011).

4.2 Tillage

Tillage is an integral part of modern agriculture that modifies the physical, chemical and biological properties of a soil. Consequently, tillage practices may also affect AM fungi (Gálvez et al. 2001; Kabir 2005; Neelam et al. 2010). The extent of extraradical AM fungal networks can be several metres per cubic centimetre of soil, providing the major nutrient–absorbing interface between plants and soil (Jakobsen et al. 1992). The persistence of AM fungi in ecosystems depends on the formation and survival of propagules (e.g. spore, hyphae and colonized roots). While spores are considered to be a resistant structure that may be viewed as long-term propagules in the absence of a viable host, hyphae are considered to be the main source of inocula for plants in undisturbed soils. The damage to these hyphal networks by tillage not only affects AM fungal growth but also reduces root colonization due to death or lowers infectivity of the hyphal fragments compared with intact hyphal networks (Johnson et al. 2001; Garcia et al. 2007).

Different AM fungi responded variedly to different tillage management practices (Gálvez et al. 2001; Kabir 2005; Borie et al. 2006). Tillage can reduce the root length colonized by AM fungi and subsequently AM-mediated P, Zn and Cu uptake by plants (Mozafar et al. 2000; Goss and de Varennes 2002). Certain AM fungal species may survive in tilled soils, while others may disappear. Because AM fungi are more abundant in the topsoil, deep ploughing may dilute their propagules in a greater volume of soil, thereby reducing their chance of association with a plant root. Soil aggregation is an important process that maintains soil porosity, hydraulic activity, organic matter and also soil erosion (Caesar-TonThat et al. 2011). But these processes are disturbed by long-term tillage systems, which subsequently not only affects the carbon stabilization and sequestration but also the microbial populations (Sainju et al. 2009). In undisturbed soil, roots follow preformed channels, making close contact with the AM-colonized root systems of the previous crop, resulting in enhanced mycorrhization of roots (Evans and Miller 1990). Furthermore, no-tillage favours the accumulation of organic matter, changes in soil structures and increased availability of C, N and water (Doran and Linn 1994; Shirani et al. 2002) in the surface horizons, thereby maximizing their benefits to crops (Kabir et al. 1999). Sheng et al. (2012) showed that long-term tillage and P fertilization invertedly affected the fine root development and AM fungal colonization in corn roots. In a recent study, Schalamuk et al. (2013) demonstrated that the effect of no-tillage or conventional tillage system on the abundance of AM fungal propagules in wheat crops depends more on the phenological stages of the crop.

4.3 Organic Manures

Organic manure consists of materials of biological origin which are used to restore the soil fertility and plant growth. According to Lee et al. (2008), general principles of organic farming include (1) exclusion of synthetic biocides; (2) addition of organic fertilizers to the soil, including farmyard manure, compost and crop residue and slow-release mineral fertilizers such as rock phosphate; and (3) use of crop rotation (IFOAM 1998). Manure application may increase or decrease root colonization by AM fungi. Tarkalson et al. (1998) found that manure application increased AM colonization, P and Zn uptake by plants and crop yield. Muthukumar and Udaiyan (2000) showed that manure applications could increase spore populations and root colonization by AM fungi. Gaur and Adholeya (2000) also found that organic amendments supported both high crop yield and AM fungal populations in onion, garlic (*Allium sativum*) and potato. The benefit of organic amendment on AM fungi has been attributed to changes in soil structure with manure amendments like increased porosity, enlarged mean weight diameter of aggregates, improved water retention capacity and greater activity of beneficial soil microbes in the soil profile (Celik et al. 2004; Pagliai et al. 2004). However, the effects of compost application on AM colonization appear to be inconsistent (Ellis et al. 1992; Allen et al. 2001). For example, the low levels of root colonization by AM fungi in soybean and sorghum in compost-amended soils were attributed to high soil P availability (Garcia et al. 2007). Muthukumar and Udaiyan (2002) showed that the growth and yield of the cowpea (*Vigna unguiculata*) varied in response with various organic amendments based on changes in indigenous AM fungal populations.

The response of plants to AM fungal inoculation in organic-amended soils has been shown to either increase (Rydlová and Vosátka 2000; Gryndler et al. 2002, 2006) or decrease (Ravnskov et al. 1999, 2006). Composted organic amendments in soil promote AM fungal hyphal growth and establishment (Douds et al. 2000), consequently aiding the transfer of mineral N (Hamel 2004) and amino acids (Govindarajulu et al. 2005) from the organic manures to the host plant. Minimal C:N ratio in the organic manures also has a positive influence on AM fungi (Groaker and Sreenivasa 1994). Likewise, the application of organic manures to soils with different nutrient levels affects the AM fungal colonization and abundance variedly. Both soil factors and N:P ratio of host roots can influence AM fungal colonization, but the mechanism remains unresolved (Liu et al. 2000; Johnson et al. 2003). Available phosphorus and mycorrhization in coconut (*Cocos nucifera*) were found to be higher in organic manure-amended soils than in inorganic fertilizer-applied soils, even though the later contain higher nutrient contents (Karunasinghe et al. 2009). The application of various combinations of organic manures (farmyard + poultry + humic acid) along with AM fungal inoculation showed enhanced plant growth and nutrient uptake and spore density in eroded soils (Sharif et al. 2012). This suggests AM fungal ability to reduce the effect of soil erosion and shield the soil fertility (Valarini et al. 2009). Numerous studies have also revealed the beneficial effects of organic manure application on AM fungi (e.g. Limonard and Ruissen 1989; Lee and Pankhurst 1992; Hole et al. 2005). Dai et al. (2011) conducted an experiment with various levels of organic amendments on chilli and showed an increased mycorrhizal colonization and a higher plant tissue nutrient in response to organic amendments.

The application of organic manures not only stimulates the AM fungal colonization of roots but also improves in spore populations in the soil. Organic manure amendment along with AM fungal inoculation has been shown to enhance plant growth and spore numbers of *C. etunicatum* and *F. mosseae* in soils than those fertilized with conventional fertilizers and inoculated with AM fungi (Douds et al. 2000). During early stages of plant growth, the spore numbers in organic manure-amended soil tend to decline and then increase subsequently due to alterations in nutrient content arising from decomposing manures (Harinikumar and Bagyaraj 1989; Muthukumar and Udaiyan 2002; Gryndler et al. 2009; Ijdo et al. 2010).

4.4 Biocides

Biocides, the chemical agents used to control pests and pathogens, are an inherent component of conventional agriculture. In plant production systems involving horticultural crops such as vegetables, most cultivators are unwilling to risk low production through reduced fertilizer or biocide inputs. Information on the effect of agricultural chemicals on AM fungi is largely empirical and poorly understood. Thus, biocide application may have inadvertent or unrecognized effects on AM fungi. Biocides used with the intention on promoting plant health may either impair or eliminate AM fungal activity causing damage to plant health. There are two beneficiary effects of biocides like fungicides on AM fungi: (a) modification of host plant physiology by enhancing root exudates that indirectly stimulate root colonization and (b) reduction of AM fungal antagonistic community (Tataranni et al. 2012). Most studies examining the effect of biocides on AM fungi are often conducted under greenhouses or in plant growth chambers, involving sterile media or media which have very little similarity to field conditions (Udaiyan et al. 1995). Soil fumigants used to reduce the abundance of pathogen cause stunting in a range of crops including onion, pepper (Hass et al. 1987), soybean (Ross and Harper 1970) and corn (Jawson et al. 1993). This reduction in plant productivity has often been attributed to decreased AM formation, which results in poor nutrient uptake. However, results of field studies do suggest that the elimination of co-occurring soil microorganisms might also substantially contribute to this effect (Hetrick et al. 1988).

Arbuscular mycorrhizal fungal responses to biocides are varied and may be influenced by the host plant, specific chemical compounds, method of application, mode of action, growth stage of AM fungi and biotic and abiotic factors (Giovannetti et al. 2006). Fungicides applied as soil drenches generally have a detrimental effect on AM fungi (Udaiyan et al. 1999). In a recent study, Rotor and Delima (2010) assessed the influence of AM fungi with the addition of N fertilizer and biocides on corn growth and productivity. The results of this study clearly suggested that microbial inoculants could act as a substitute for biocide application. But the effect of fungicides on AM association can vary with host–fungal combinations. For example, the dicarboximide fungicide captan is known to stimulate mycorrhization of beans by *Glomus* spp. (De Bertoldi et al. 1977), had no effect on undetermined species colonizing onion (El-Giahami et al. 1976) and reduced colonization by *F. mosseae* in corn (Sutton and Sheppard 1976). Fungicides can also adversely affect different stages of AM fungal development and function (Trappe et al. 1984). The influence of three commonly used fungicides, i.e. benomyl, pentachloronitrobenzene, and captan, tested on mixed culture of AM fungi indicated that these fungicides could alter the species composition of AM fungal community (Schreiner and Bethlenfalvay 1996). Nevertheless, the biological response of AM to these fungicides depends not only on the fungus–fungicide relationship but also on the prevailing environmental conditions (Schreiner and Bethlenfalvay 1997). Systemic fungicides like carbendazim can completely inhibit P uptake by AM fungal hyphae even when applied at recommended field rates (Kling and Jakobsen 1997). In addition, carbendazim could disrupt hyphal P uptake at concentrations as low as 10 % of the recommended field dosage (Schweiger and Jakobsen 1998). However, under field conditions, carbendazim or a mixture of propiconazole and fenpropimorph applied at recommended rate did not affect AM colonization (Schweiger et al. 2001). In contrast to carbendazim, the benzimidazole fungicide benomyl has to be applied at a much higher rate than recommended levels to affect AM fungal colonization of roots (Gange et al. 1993).

Like certain fungicides, higher dosage of pesticides like malathion and mancozeb reduces plant growth parameters and also affects the extent of mycorrhizal colonization (Saleh Al-Garni 2006). Unlike other pesticides, the herbicide atrazine at lower concentration decreases mycorrhizal colonization, whereas at higher concentration, it stimulates colonization (Huang et al. 2006, 2007). However, it has been speculated that the application of higher concentrations of atrazine tends to induce tolerance in AM fungi, although the real mechanism behind the varied effect is yet to be elucidated (Huang et al. 2007).

4.5 Crop Rotation

In general, microbes in the soil affect the succeeding crop. From the biological view point, crop rotation is essential for proliferation of AM fungi (Douds et al. 2005). The AM fungi which proliferate with a host plant are not necessarily those best at promoting the growth of other crops in the rotation (Feldmann et al. 1999). The proliferation of such AM fungi has been attributed as a cause to yield decline in continuous monoculture (Schenck et al. 1989). This had been noted in soybean and sorghum grown as continuous cropping and also by crop rotation, which reflected less mycorrhizal colonization in the former method than the later (Ellis et al. 1992). In addition, the diversity of the AM fungal community is linked to the diversity and productivity of the plant community (van der Heijden et al. 1998; Bever et al. 2001). A more relaxed altitude towards weed management may increase both the diversity and effectiveness of the AM fungal community when the crops are non-mycorrhizal (Miller and Jackson 1998; Feldmann and Boyle 1999; Jordan et al. 2000). This is important under circumstances where the cultivation of non-host crop such as *Brassica* spp. is known to reduce AM fungal inoculum in the soil (Blaszkowski 1995).

Pre-cropping enhances mycorrhizal inoculum potential (Dodd et al. 1990a, b; Karasawa et al. 2001, 2002). The enhancement of mycorrhiza inoculum potential by a given pre-crop may improve the mycorrhizal activity of a subsequent crop in the rotation (Barea et al. 1993). This is because the fungi develop and sporulate mostly in the roots of those plant species, which are most susceptible to mycorrhizal colonization. Susceptible crops, which in the rotation follow non-host plants (or plants, which develop little mycorrhizal colonization), may carry less colonization than they would follow a strongly mycorrhizal crop (Ocampo and Hayman 1981). The composition of AM fungal spore communities tends to change significantly if crop rotation was practiced along with P fertilization (Mathimaran et al. 2007). According to Vestberg et al. (2005), improved P nutrition of rice grown under acidic and phosphorus deficiency conditions without using P fertilizer could be achieved by crop rotation (maize and horse gram) along with AM fungal inoculation.

4.6 Fallow Period

Fallow, the reinstatement period to trim down the weed growth, may negatively influence AM fungi. Crops including corn, sorghum, sunflower, chickpea and linseed, when grown in southern Queensland, Australia, after long periods of bare fallow, exhibited poor growth with P and Zn deficiency. This syndrome, termed long-fallow disorder, was associated with low AM colonization, failure of AM fungal mycelia networks in soil to take up sufficient nutrients and reduced AM fungal spore density and diversity (Kabir and Koide 2000; Karasawa et al. 2002). The application of P and Zn fertilizers to soils following long fallow not only alleviated the negative effects of low AM colonization and fertility, but crops responded better than in soils with higher AM fungal inoculum levels (Thingstrup et al. 1998). Poor growth of linseed after prolonged fallowing in a semiarid cropping system was improved by inoculation with AM fungal propagules obtained from sorghum-cropped field soil (Thompson 1994). A 1-year fallow in an oxisol reduced the number of AM fungal propagules by 40 % and the growing of non-mycorrhizal crops like mustard (*Brassica juncea*) reduced them by 13 % (Harinikumar and Bagyaraj 1988). Wagner et al. (2001) observed an exponential decline in spore counts of *Claroideoglomus claroideum* (=*Glomus claroideum*) with time during soil storage. with time during soil storage. Ellis (1998) also showed that the absence of host roots could drastically reduce AM fungal populations in the soil. The findings of Troeh and Loynachan (2003) suggest that continuous cropping of maize and soybean increases AM fungal spore numbers, whereas spore numbers tend to decrease under fallow. Recently, Karasawa and Takebe (2012) reported that fallow condition could reduce the abundance of AM fungal propagules (spores and mycelium), due to the disruption of AM fungal mycelial network and alteration of available nutrients and microbial activities in soil (Jansa et al. 2003). To overcome the defects, the maintenance of high AM fungal abundance in cropping systems could ensure tolerance to prolonged fallow periods and their activity (Hijri et al. 2006).

4.7 Management Considerations

Reports of improved plant growth responses in response to AM inoculation under controlled conditions in low fertile soils led to a flurry of activities during 1980s, aimed at utilizing AM fungi as bioinoculants. However, the magnitude of responses was different under field conditions, especially under conventional high-input agricultural systems. Further studies, however, have shown that most crop species are mycorrhizal and AM fungi can have a substantial positive or negative impact on crop productivity (Johnson et al. 1997). Therefore, there is a need to elucidate the role of AM fungi in agroecosytems and to understand the impact of management practices on the symbiosis. The introduction of appropriate fungi to the plant production systems may be appropriate under conditions where the native AM fungal inoculum potential is low or inefficient. The initial step in any inoculation programme is to identify and isolate organisms that are both infective (able to associate) and effective (able to impart desired effects) under a given set of conditions. Isolates of AM fungi may vary widely in these properties. So, screening trials are needed to select isolates that will perform efficiently and successfully. Screening under actual field conditions is preferred than under controlled conditions, because the influence of indigenous AM fungi, soil organisms and cultural practices on the introduced fungi could be more clearly understood. Factors that should be considered when assessing the potential role and introduction of AM fungi in agroecosystems include:

4.7.1 Mycorrhizal Growth Response (MGR) and Mycorrhizal Dependency (MD) of the Host Crop

Mycorrhizal growth response (MGR) is the responsiveness of change in the total biomass of mycorrhizal (M) versus non-mycorrhizal (NM) crop plants from the symbiosis (Hetrick et al. 1992):

$$\mathrm{MGR} = \left[(\mathrm{M}-\mathrm{NM})/\mathrm{NM}\right]\times 100$$

Mycorrhizal dependency (MD) is defined as the growth response of the total dry matter in mycorrhizal (M) versus non-mycorrhizal (NM) plants at a given phosphorus level (Plenchette et al. 1983):

$$\mathrm{MD} = \left[(\mathrm{M}-\mathrm{NM})/\mathrm{M}\right]\times 100$$

All mycorrhizal agricultural crops are not equally benefitted from the association. Generally, coarse-rooted plants like legumes benefit more from AM symbiosis than fine-rooted cereals (Jeffries and Dodd 1991). Mycorrhizal dependency of a crop species may differ with the cultivars as well as with the AM fungal species involved (Table 2; Figs. 5 and 6).

4.7.2 Inoculum Density, Rate and Extent of AM Colonization

Rapid and extensive spread of AM fungal colonization is a crucial factor for effectively enhancing plant growth and ably competing with indigenous AM fungi. Therefore, the formation of entry points is important, and their number is controlled by inoculum level, more specifically by inoculum density, that is, the number of propagules per given unit of soil. Experiments are preformed either with unknown quantities of AM fungal inoculum or state only the spore numbers. However, the spore numbers alone do not constitute total propagules, as dried root bits, sporocarps, soil hyphae and mycorrhizal roots can also act as propagules. Therefore, currently, the total

Table 2 Mycorrhizal dependency of agricultural crops under low soil P

Mycorrhizal dependency	Agricultural crops
Strong	Cassava, onion, legumes, peppers
Medium	Soya, wheat, barley, cowpea, grain legumes, tomatoes
Weak	Potato, rice, melon, sunflower, beans, maize, sorghum

After Jeffries and Dodd (1991) with permission

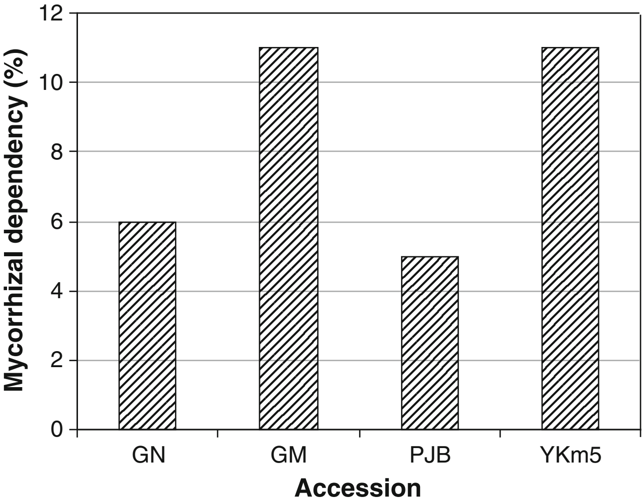

Fig. 5 Relative mycorrhizal dependency of four *Musa* cultivars (*GN* Grande Naine, *GM* Gros Michel, *PJB*, Pisang Jari Buaya, *YKm5*, Yangambi Km5) inoculated with *Funneliformis mosseae* (Data from Elsen et al. 2003, with permission)

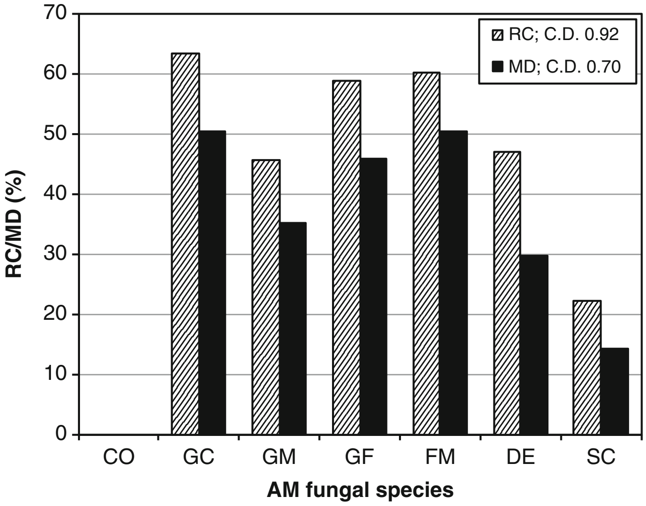

Fig. 6 Effect of different arbuscular mycorrhizal fungi on root colonization (RC) and mycorrhizal dependency (MD) of *Eleusine coracana* var. HR-374. *CO* Control, *GC Glomus caledonium*, *GM Gigaspora margarita*, *GF Glomus fasciculatum*, *FM Funneliformis mosseae, DE Diversispora epigaea* (=*Glomus epigaeum*), *SC Scutellospora calospora*, *C.D* critical difference ($P < 0.05$) (Data from Tewari et al. 1993, with permission)

number of propagules available for colonization is used to measure the AM fungal propagule density in field soil or inoculum (Muthukumar and Udaiyan 2003). For example, the reduction in plant growth had been shown under field conditions under reduced spore density and root colonization (Zangaro et al. 2008, 2012). Similarly, comparisons between different AM fungi should be made at similar inoculum densities. A study by Rajan et al. (2000) showed that the infectivity and rate of colonization development of *Gigaspora margarita* were greater than those of *Rhizophagus intraradices* (=*Glomus intraradices*). Likewise, propagules of *F. mosseae* were found to be most effective compared to those of *R. intraradices* (Rajan et al. 2000). Studies have also shown that inoculum consortia of AM fungi perform better than inoculum containing single taxa (Khade and Rodriguez 2009). In a recent study, Jin et al. (2013) using plant growth parameters and molecular techniques to detect the presence of AM fungi within plant roots showed that a mixed culture [*Rhizophagus irregularis* (=*Glomus irregulare*), *F. mosseae* and *Rhizophagus clarus* (=*Glomus clarum*)] functioned better than a single species (*R. irregularis*) inoculation in field-grown pea plant.

4.7.3 Efficiency of Inocula

For field applications, it is always essential to confirm that the inoculated microorganisms possess all the qualitative characteristics of an inoculum. The efficiency of the inoculum primarily depends on the performance of the AM fungal strain adapted to the host plants for their establishment. However, this tends to vary with the fungus and the host plants. To characterize the efficiency of inoculum, various approaches including the identification of the spore, estimation of root fungal colonization (Dalpé 1993) and an assessment on spore germination rate are often used. Further, molecular techniques are also handy for the detection of the introduced AM fungal strain among the indigenous AM fungal strains naturally occurring in the soil. These techniques are not consistent with morphological identification, due to the large genetic variability in AM fungi, and these techniques are rarely used. Consequently, internal transcribed spacer (ITS) sequences of ribosomal DNA genes (rDNA) were also used in some discrimination. Current researches focus on the development of consistent molecular techniques to trace the inoculated strains using PCR and species-specific primers (Séguin et al. 2003) and also for the entrapment of AM fungal propagules in natural polysaccharide gels (Vassilev et al. 2005; Siddiqui and Kataoka 2011) are under study. Such technological breakthroughs would greatly facilitate both fundamental and applied researches on mycorrhizae as well as improve quality control of commercial inocula.

5 Methods of Inoculum Production

The production of inoculum is more essential for AM fungi due to its obligate symbiotic nature. Traditionally, AM fungi are propagated through open pot cultures. Starter cultures are usually initiated from spores or colonized root fragments, which are later incorporated into the growing medium for seedling production (Brundrett et al. 1996). The fungi spread in the substrate and colonize the roots of the seedlings. Both colonized substrates and roots can then serve as mycorrhizal inoculum. Soilless culture systems such as aeroponic cultures enable the production of cleaner spores and facilitate uniform nutrition of colonized plants (Singh et al. 2012). The successful propagation of some AM fungal strains on root-organ cultures has facilitated the development of monoxenic strains that can be used directly as inoculum for in vitro plant production systems or for large-scale inoculum production (Fortin et al. 2002).

5.1 Off-Farm Methods

The various advantages and disadvantages of different off-farm inoculum production methods are shown in Table 3. Highly infective soil-based inocula are quite easy to produce and handle. However, the time span required to produce appreciable quantities of soil-based inocula can range between 6 and 12 months. An inoculum form produced using light expanded clay aggregates as substrate is of interest (Dehne and Backhaus 1986), as the porous material containing infective mycelium and spores can be easily separated from the plant roots (Feldmann and Idczak 1992). The aggregates can later be surface sterilized and applied to field-grown crops in small quantities (Baltruschat 1987). Vermiculite is an inorganic carrier for AM inoculum production, as it is an ideal substrate for AM fungal sporulation (Barea et al. 1993). Sheared-root inocula (Sylvia and Jarstfer 1992), prepared from aeroponic cultures (Hung and Sylvia 1988; Jarstfer and Sylvia 1999), are also routinely used in inoculation programmes. Surface-disinfected AM fungal propagules can also be used especially under in vitro conditions. Two methods of off-farm inoculum production (open pot culture and root-organ culture) are detailed below.

Table 3 Arbuscular mycorrhizal fungal inoculum types with their advantages and disadvantages

Inoculum type	Advantages	Disadvantages
Soil based	Long shelf life	Bulk and heavy
	Useful at transplanting	Needs soil sterilization
Soilless substrate	Weightless than soil Uniform composition Can be dried and stored	Needs careful control of watering and fertilizer application
Surface-sterilized AM propagules	Aeroponically developed colonized roots can be sheared	Needs highly skilled technique
Entrapment in polymer gel, alginate, hydrogel, hydroponic, aeroponic and root-organ culture	Can be kept free of extraneous organisms Can be dried and stored	Relatively expensive

Modified from Azcon-Aguilar and Barea (1997) with permission

5.1.1 Open Pot-Culture Production

Arbuscular mycorrhizal fungi are obligate symbionts, which require a suitable host plant for its establishment and proliferation. Conditions for large-scale inoculum production need to be optimized. Care should be taken to identify and avoid contamination from undesired species during monoculture. The process to attain huge amount of targeted inoculum is rather lengthy. The inoculum production through open pot cultures involves the following steps.

5.1.1.1 Isolation of AM Fungal Strain

Spores of desired AM fungal strain are collected from the plant's rhizosphere by wet-sieving and decanting technique (Gerdemann and Nicolson 1963). However, for raising monoculture, AM fungal propagules can be acquired from trap cultures. The trap culture constitutes the host plant rhizospheric soil diluted equally with sterilized sand (Menge 1984). The resulting AM fungal spore densities are higher compared to the initial rhizosphere soil. In fact, undetected spores in the initial extraction of field soil could be detected in trap culture (Mortan et al. 1995). Liu and Wang (2003) evaluated the presence of AM fungal spores by using different trap plants. The results of this study suggested that the numbers of AM fungal spores and species were higher in trap cultures and clover (*Trifolium repens*) was found to be a suitable host for identifying the AM fungal diversity. Using spores for initiating cultures certainly has some benefits like easy detection of undesired fungal spores, quick enumeration and evaluation of spore viability and germination and a reduction of pathogen inclusion (Dodd and Thomson 1994). On the other side, AM fungal spores sometimes exhibit dormancy, which reduces the potential of the inoculum (Gemma and Koske 1988).

5.1.1.2 Choice of a Host Plant

The selection of a suitable host plant should be based on high mycorrhizal dependency, adaptability to in vitro or greenhouse conditions and in having an extensive root system. The host plants commonly used for raising pot cultures are corn, onion, leek (*Allium porrum*), *Sorghum halepense*, Bahia grass (*Paspalum notatum*), Guinea grass (*Panicum maximum*), buffell grass (*Cenchrus ciliaris*) and subterranean clover (*Trifolium subterraneum*) (Chellappan et al. 2001). Generally, monocots are preferred as hosts, because the fibrous root system enables uniform spreading of the roots in a given volume of soil than plants with tap roots. Further, the fibrous root system renders monocots less dependent on mycorrhizal fungi than dicots.

5.1.1.3 Optimizing Growing Conditions

Properly sterilized substrate is essential not only to maintain the purity of a culture but also for avoidance of diseases. Usually, equal proportions of sterilized sand soil mixture are used for raising inoculum (1:1; sand/soil). A coarse-textured sandy soil (Gaur and Adholeya 2000) mixed with vermiculite or perlite or turface (Dehne and Backhaus 1986) can also be used. Inadequate mineral nutrient in the substrate may affect plant and in turn the fungal development. However, the excess of available P can inhibit AM fungal propagation. As N, K, Mg and microelement ratios may affect AM inoculum development, plant fertilization needs to be performed artificially especially when inert substrates are used for inoculum production (Dixon et al. 1999). In addition, other edaphic and climatic factors such as pH, soil temperature and aeration, light intensity and relative humidity need to be controlled for optimal AM fungal propagation (Rao and Tarafdar 1999). Some disadvantages of open pot-culture production include bulkiness, transportation problems, cross-contamination and lack of genetic stability (Abdul-Khaliq et al. 2001).

5.1.2 In Vitro Propagation on Root-Organ Culture

The root-organ culture involves the proliferation of excised roots under axenic conditions on an artificial nutrient media supplemented with vitamins, minerals and carbohydrates. This method was first used for in vitro AM fungal propagation by Mosse and Hepper (1975). Root-organ cultures with vigorous root formation and uniform growth under poor nutrient conditions (alteration in hormones) have been obtained through the transformation of roots by the soil bacterium *Agrobacterium rhizogenes* (Abdul-Khaliq et al. 2001). Nevertheless, when hairy root technique started to emerge, AM fungal propagules like spores or sporocarps, mycorrhizal root bits and even isolated vesicles were used in hairy root cultures to initiate in vitro AM fungal inoculum

(Kapoor et al. 2008). Surface sterilization of AM fungal spores is done using various sterilizing agents like chloramine-T or Tween 20 (Fortin et al. 2002) followed by antibiotic solution wash to remove contaminants from spore surfaces. All the above said processes need to be carried at reduced temperatures. The selection of culture medium is of prime importance, because both roots and AM fungal propagules require different media compositions for their growth. The negative geotropic nature of transformed roots facilitates its contact with the AM fungal hyphae, thereby initiating colonization. Recently, Abd-Ellatif et al. (2012) demonstrated the successful establishment of AM fungal association in root-organ culture using tomato hairy roots.

5.2 On-Farm Methods

On-farm production of AM fungal inoculum entails increasing the propagules of desired isolates and indigenous AM fungi in fumigated and unfumigated field soils, respectively, or transplanting pre-colonized host plants into compost-based substrate (Douds et al. 2005). This type of AM fungal inoculum production would enable farmers to obtain inoculum at a cheaper cost and make their transportation easy. Furthermore, farmers could easily produce locally adopted isolates and generate a taxonomically diverse inoculum in large quantities.

5.2.1 Method 1

The earliest method of inoculum production for an effective strain of the AM fungus *Rhizophagus manihotis* (= *Glomus manihotis*) was developed by Sieverding (1987, 1991) in Columbia. In this method, first, a 25 m^2 field plot was tilled and fumigated to eliminate the indigenous AM flora. After the fumigant has dissipated, the inoculum of the specific AM fungal strain (*R. manihotis*) was inoculated into holes drilled in the soil and then seeded with a grass host, *Brachiaria decumbens*. Simultaneously, pre-colonized *B. decumbens* plants were also transplanted to the inoculum preparation plots, thereby minimizing the amount of starter inoculum needed. After 4 months of growth, the soil and roots were harvested to a depth of 20 cm and used as inoculum.

5.2.1.1 Advantage

A postharvest analysis of the inoculum showed that fumigation of the soil was essential to increase the AM fungus spore production per given quantity of the soil. Further, fumigation also increased the relative proportion of spores of the desired AM fungal isolate relative to indigenous AM fungi compared to unfumigated and inoculated plots.

5.2.2 Method 2

The second method of on-farm AM fungal inoculum production involves preparing raised soil beds (60 × 60 × 16 cm) (Gaur 1997; Douds et al. 2000). After fumigation of the beds, the AM fungi from a starter inoculum were inoculated into furrows in the raised beds. A succession of hosts [(e.g. *Sorghum sudanese*, corn and carrot (*Daucus carota*)] were grown for 1 year of 4 months each. The growth cycle was carried over a course of 3 years. After the third cycle started to progress, the soil in raised beds was found to be ready to be used as inoculum.

5.2.2.1 Advantage

An approximately tenfold increase in AM fungal inocula was evident from year 1 to year 3, yielding around 2.5×10^6 propagules per bed.

5.2.3 Method 3

Gaur et al. (2000) and Gaur and Adholeya (2002) later modified the above method to yield a shorter inoculum production cycle without the use of fumigants. Raised beds were prepared as stated above (method 2) by using 2:1 (v/v) mixture of soil to leaf compost. The beds were either inoculated or left uninoculated to enhance the proliferation of indigenous AM fungi. In this method, only one plant growth cycle was used involving forage crops or vegetables as host plants.

5.2.3.1 Advantage

In this method, inoculum production was 15- to 20-fold greater than the starter inoculum used. This method produced only 55–69,000 propagules per bed, 40-fold lower than the 3-year method (method 2).

5.2.4 Method 4

In 2005, Douds et al. developed another method for on-farm production of AM fungal inoculum for temperate regions. Raised bed enclosures (0.75 × 3.25 × 0.3 m) were constructed with silt fence walls, weed barrier cloth floors and plastic sheeting dividing walls dividing the enclosure into 0.75 m square sections. The enclosures were filled to a depth of 20 cm with a 1:4 (v/v) mixture of compost and vermiculite. Pre-colonized ten Bahia grass plants were transplanted into the enclosures. One AM fungal isolate was used per enclosure section. The enclosures were tended for one growing season and watered as needed (Douds et al. 2005).

5.2.4.1 Advantage

The advantage of this method includes the production of significant quantities of the desired AM fungi. An average of 95×10^6 propagules could be produced per 0.75 × 0.75 m enclosure section.

5.2.5 Method 5

In 2006, Douds et al. suggested another method for on-farm production of AM fungus inoculum. Bahia grass seedlings colonized by AM fungi were transplanted into raised bed enclosures consisting of vermiculite mixed with either field soil or yard clipping compost or vermiculite mixed with yard clipping compost or dairy manure/leaf compost. The propagule yield was higher in compost and vermiculite mixture compared to soil-based mixture. Inoculum production in a 1:4 (v/v) mixture of yard clipping compost and vermiculite media was more (503 propagules cm^{-3}) than those with 1:9 and 1:99 (v/v) mixtures (240 and 42 propagules cm^{-3}), respectively (Douds et al. 2006).

5.2.5.1 Advantage

This method enables the production of concentrated AM fungal inoculum that can be readily used in horticultural potting media for vegetable seedling production. Supplemental nutrient additions are unnecessary during inoculum production.

5.2.6 Method 6

In 2010, Douds et al. modified the existing method for the production of AM fungal inoculum in temperate climates. Black plastic bags filled with approximately 20 L of a 1:4 (v/v) mixture of pasteurized compost and vermiculite served as the growing medium. To this growing medium, field soil (containing 12 propagules cm^{-3}) collected from the top 10 cm from a field was mixed at the rate of 100, 200 or 400 cm^3. Three-month-old non-mycorrhizal or mycorrhizal Bahia grass seedlings were planted in the bags at a rate of five plants per bag and grown for 3 months. Adding 100 cm^3 of field soil to the growing medium and planting with non-mycorrhizal seedlings produced 465 propagules cm^{-3} compared to 137 propagules cm^{-3} for planting with the pre-colonized seedlings (Douds et al. 2010).

5.2.6.1 Advantage

This modification to the existing method allows greater flexibility and makes it easier for the production of the AM fungal inoculum directly on the farm. This method could be readily adopted by farmers.

6 Determination of Infective Propagule Abundance

The most suitable and convenient method to determine the number of infective propagules of AM fungi in a crude inoculum, soil or mycorrhizal root bits is termed as the most probable number (MPN) technique (Alexander 1982). It has been assessed by using a statistical estimation of microbial population density (Cochran 1950).

6.1 Inoculum Management

Inoculum formulation procedure consists of placing fungal propagules (root fragments, mycelium and spores) in a carrier (perlite, peat, inorganic clay, zeolite, vermiculite, sand, etc.) for a given application (Gianinazzi and Vosátka 2004). The critical factor that determines the inoculum efficiency is the dosage and the time of inoculation. Although, theoretically, a single propagule of AM fungi is sufficient to initiate mycorrhization, the colonization process in such cases is very slow to be of agronomic interest. About 1–2 kg of soil inoculum (with 5,000–10,000 propagules) per m^2 of seedbed could be an appropriate application rate. However, the application rate for each crop species has to be standardized for a given set of environmental conditions (Muthukumar and Udaiyan 2003). The time of inoculation is also important, and in general, the earlier the inoculation, the greater the benefit to the plant (Barea et al. 1993).

6.2 Inoculation Methods

The aim of inoculation is to introduce desired AM fungal propagules into the rhizosphere of the target plant (Jarstfer and Sylvia 1992). Various methods of AM fungal inoculation for transplanted and field-sown crops have been detailed by Bagyaraj (1992). The most common method is to place the inoculum below the seed or seedling, prior to seeding or planting. Seedlings raised in sterilized or unsterilized nursery beds or containers containing selected AM fungi can be transplanted after mycorrhizal association is well established. This method has been successfully used for agronomic crops like chilli, finger millet, tomato and tobacco (*Nicotina tabacum*) (Govinda Rao et al. 1983; Sreeramulu and Bagyaraj 1986). For field-sown crops, AM fungi can be applied as seed coating (Hattings and Gerdemann 1975), mycorrhizal pellets (Hayman et al. 1981; Hall and Kelson 1981), fluid drilling (Hayman et al. 1981) and inoculation in furrows (Hayman et al. 1981; Powell and Bagyaraj 1982).

7 Conclusion

The role of AM fungi in enhancing plant growth is proved beyond doubt both under on-field and off-field conditions. Responses to AM fungal association are most widely to occur when mycorrhizal-dependent crop species are raised on substrates with low P levels. Therefore, it is important to determine the mycorrhizal dependency of the crops grown in a region and to select those which could respond to AM inoculation. Screening for selection of an efficient crop–AM fungal combination should be undertaken. Research should be intensified in the direction of manipulating AM fungi in the indigenous AM fungal community to achieve maximum crop productivity. The optimization of agronomic practices, reducing fertilizer input and use of cheap source of fertilizer (rock phosphate, organic manures) should be investigated. Top priority should be given to the development of new technologies for rapid and large-scale quality inoculum production. Further, one of the main tasks in AM research is to raise awareness in the growers' mind about the potentials of AM technology in sustainable crop production and soil conservation.

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Recycled Water Irrigation in Australia

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Abstract

Access to water has been identified as one of the most limiting factors to economic growth in Australia's horticultural sector. Water reclaimed from wastewater (sewage) is being increasingly recognised as an important resource, and the agricultural sector is currently the largest consumer of this resource. An overview of the Australian experience of using reclaimed wastewater to grow horticultural crops is presented in this chapter. The wastewater treatment process and governing regulations are discussed in relation to risk minimisation practices which ensure that this resource is used in a sustainable manner without impacting adversely on human health or the environment. A case study covering the socio-economic and environmental implications of recycled water irrigation is also presented.

Keywords

Wastewater • Agriculture • Irrigation • Water recycling • Water treatment • Nutrients • Contaminants • Sodicity • Salinity • Best practice management

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1 Introduction

In Australia, the agricultural sector occupies approximately 54 % of the land and is the largest consumer of water. In 2008–2009, the total water use by the agricultural sector was 6,696 GL, 47 % of the total for Australia. In the northern regions where rainfall is more reliable, the predominant enterprises are beef cattle grazing, sugar and tropical fruit farming. In the south where summers are generally dry, dry land cereal farming, sheep grazing and dairy farming (in areas of higher rainfall) predominate (ABS 2010a). Whilst the gross value of irrigated agricultural production in 2006–2007 was 34 % of the total gross value of agricultural production, irrigated farmland represents only 1 % of the total land used for agriculture; most of this land is within the confines of the Murray-Darling Basin (ABS 2010a).

Policy changes to return environmental flows to the rivers, coupled with 8–10 years of drought, have seen water allocations decline, placing increasing pressure on irrigators. Despite water conservation steps across several states which have seen a 43 % reduction in water consumption throughout the country over the period of the drought (from 24,909 GL in 2000–2001 to 14,101 GL in 2008–2009), irrigators are still challenged with ongoing reductions in water allocations (ABS 2010b). In the face of these shortages, water reclaimed from wastewater is being increasingly recognised as an important resource which provides benefits for the community and the environment by increasing available water resources, returning critical environmental flows to failing waterways and decreasing nutrient and contaminant loads to surface and coastal waters.

"Treated" sewage water (commonly known as wastewater, recycled water or reclaimed water) has been underutilised in Australia, although increases in its reuse have been seen since the mid-1990s (Anderson and Davis 2006). There has been a steady increase in the volume of water reuse in agriculture, and currently around 11.5 % of total wastewater generated is reused (ABS 2010b). Although agriculture uses the largest amount of recycled water (103 GL/year), this represents only 1 % of the total volume of water used by the agricultural sector (ABS 2010b). Figure 1 shows both the distribution of reuse water consumption throughout the agricultural

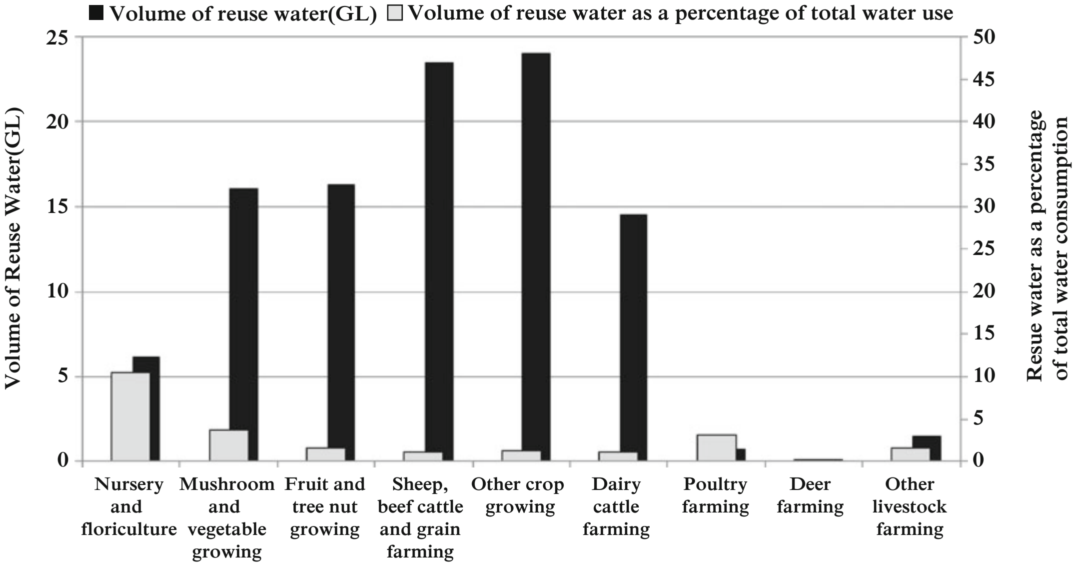

Fig. 1 Reuse water consumption within the Australian agricultural sector and as a percentage of total water consumption (From ABS 2010b)

sector and the reuse water consumption as a percentage of total water use for each enterprise type. Whilst beef, sheep and grain production use the largest volume of reuse water, it amounts to only 1 % of this industries' total water use (ABS 2010b). Horticulture and floriculture use the greatest amount as a proportion of total water use (10 %), followed by vegetable and mushroom production (4 %). This may be a reflection of the relative proximity of these industries to major wastewater treatment plants which supply the majority of reuse water and are located in proximity to densely populated urban areas.

2 Wastewater Treatment and Public Health

2.1 Wastewater Treatment

Untreated wastewater originates from domestic households, commercial premises and industrial activities. It does not include storm water which is the rainfall run-off from sealed surfaces including roofs and roads. It typically consists of 99.9 % water and 0.1 % impurities which include: dissolved and suspended organics, pathogens, nutrients, trace elements, salts, refractory organics, priority pollutants and heavy metal(loid)s. Varying degrees of treatment can be applied to remove or reduce these contaminants in wastewater. The aim of wastewater treatment is to produce water which is fit for purpose, i.e. when used as intended will not threaten human health or degrade the receiving environment. The extent of treatment required is usually regulated by Public Health or Environmental Protection Authorities.

Wastewater reclamation processes are traditionally grouped into preliminary, primary, secondary and tertiary treatment processes though it is possible to find significant overlap at wastewater treatment plants. At the preliminary stage, large debris and finer abrasive material are removed to prevent damage to downstream equipment. In the primary processes, sedimentation is used to remove approximately 65 % of the solid content of raw sewage and approximately 35 % of the biochemical oxygen demand (BOD). Sedimentation also removes a proportion of the heavy metal(loid)s which, being mainly cations, bind to negatively charged organic matter and clay particles. In secondary processes, bacteria remove soluble and colloidal wastes by assimilating organic matter to form new microbial biomass and by producing gas through the use of organic matter for endogenous respiration. Tertiary processes such as nutrient removal, filtration and disinfection are employed as an additional step to achieve sufficient removal of coliforms, parasites, salts, trace organics and heavy metal(loid)s to make the water suitable for unrestricted irrigation of food crops. These steps can be carried out concomitantly with earlier processes and include nutrient removal through precipitation (as in the case of phosphorus-P) and gaseous emission (as in the case of nitrogen-N though denitrification), and disinfection by UV light, chlorine or ozone reduces the number of pathogens present in the waste stream by inactivation.

2.2 Governance and Quality Requirements of Reclaimed Water Use

Regulations governing the use of reclaimed water are not uniform throughout Australia; each state and territory has responsibility for managing natural resources and public health in their jurisdiction. Legislation for wastewater reuse is covered by acts relating to food safety, public health and/or environmental protection. As such the state Public Health Authority and/or Environmental Protection Agency have responsibility for policing reclaimed water reuse.

Many states require enterprises which irrigate with reclaimed wastewater or supply reclaimed wastewater for the purpose of irrigation to produce and adhere to environmental (irrigation) management plans and/or user agreements. The plans should include a study of the irrigation site characteristics and justify how the wastewater will be applied so that its use will not threaten human health or adversely impact on the receiving environment. The need for user agreements to ensure wastewater is being utilised in the

Table 1 Water quality standards and applications for water classes A–D in South Australia

Class	Applications	Microbiological criteria	Chemical/physical criteria
A	Primary contact recreation	<10 *Escherichia coli*/100 mL	Turbidity ≤2 NTU
	Residential non-potable	Specific removal of viruses, protozoa and Helminths may be required	BOD <20 mg/L
	Unrestricted crop irrigation		Chemical content to match use
	Dust suppression with unrestricted access		
	Municipal use with public access		
B	Secondary Contact recreation	<100 *Escherichia coli*/100 mL	BOD <20 mg/L
	Restricted crop irrigation	Specific removal of viruses, protozoa and Helminths may be required	Suspended Solids <30 mg/L
	Irrigation of pasture and fodder for grazing animals		Chemical content to match use
	Dust suppression with restricted access		
	Municipal use with restricted access		
C	Passive recreation	<1000 *Escherichia coli*/100 mL	BOD <20 mg/L
	Municipal use with restricted access	Specific removal of viruses, protozoa and Helminths may be required	Suspended Solids <30 mg/L
	Restricted crop irrigation		Chemical content to match use
	Irrigation of pasture and fodder for grazing animals		
D	Restricted crop irrigation	<10000 *Escherichia coli*/100 mL	Chemical content to match use
	Irrigation for turf production	Helminths may need to be considered for pasture and fodder	
	Silviculture		

Source: DOH and EPA (1999); *NTU* Nephelometric Turbidity Units

approved manner varies across the jurisdictions as do the requirements for ongoing monitoring, audits and reviews. The extent of the relevant authorities' ongoing involvement in a scheme depends on its size, the risk associated with reuse and sensitivity of the receiving area.

Currently each state authority holds the responsibility for defining the quality of water that can be used to irrigate fruits/vegetables; guidelines for reclaimed water use exist in each state and territory (Power 2010). Recycled water guidelines set targets for removal of pathogens, nutrients, toxicants and salts. Health-based targets receive the greatest emphasis, and microbial contaminants present the greatest risk to human health; studies have shown that in achieving targets for pathogen removal, the chemical hazards which threaten human health are also reduced to acceptable levels.

Both the National and State guidelines for recycled water use were until recent times based around matching defined classes of water (based largely on their pathogen burden, biochemical oxygen demand and turbidity) with preapproved uses (Table 1). The highest quality A^+ recycled water could be used in residential dual reticulation systems, and the lowest classes, C or D, could only be used for irrigation of nonfood crops, e.g. instant turf, woodlots and flowers.

In 2006, in the face of increasing pressure on freshwater resources, the National Water Quality Management Strategy *Australian Guidelines for Water Recycling*: *Managing Health and Environmental Risks* (AGWR) was released (NRMMCEP and HCAHMC 2006). The AGWR was produced in an effort to establish consistent standards for reclaimed water schemes across the country and to introduce the risk management framework promoted by the *World Health Organization's Guidelines for Drinking-water Quality* (WHO 2008).

The AGWR does away with the class-based system and advocates a risk assessment-based approach. Each scheme is individually assessed; water quality targets, treatment processes and additional preventative measures are tailored to produce a safety level consistent with the proposed end use of the reclaimed water. The emphasis is no longer on end of line testing but on developing a multi-barrier approach to reduce risk to an acceptable level known as the "tolerable risk". The risk assessment is carried out largely in relation to hazards to human health, and in this regard microbial pathogens are the greatest threat (NRMMCEP and HCAHMC 2006). The AGWR national guidelines are not mandatory, and several states have elected not to adopt the new approach at this point in time (Power 2010).

3 Key Environmental Hazards

The contaminants in reclaimed water that present the greatest risk to the receiving environment include boron (B), cadmium (Cd), chlorine (Cl) and disinfection residuals, N, P, Sodium (Na) and Chloride.

3.1 Nutrients

Human and domestic wastes contribute large amounts of N and P to sewage. Only 50 % of the N and 60 % of the P are removed during treatment so the concentration of these major nutrients still remains higher in treated sewage than in irrigation water from other sources (Kelly et al. 2006).

Whilst the use of treated wastewater can benefit crop nutrient management, application in excess can be detrimental to both crops and the local environment. The nutrient load supplied to a crop is determined by the nutrient concentration of the reclaimed water and the irrigation depth (Kelly et al. 2006). Table 2 outlines the macronutrient uptake of a range of vegetables and the nutrient load supplied by an irrigation depth of 1,000 mm from wastewater which is treated to a tertiary level; the data demonstrates that at this level of irrigation, some nutrients would be supplied in excess of requirements, thereby likely to result in the loss of nutrients through leaching and surface run-off.

Nitrate is the most mobile form in soil and can be subject to leaching if nitrate and water are applied in excess of the plants' needs, this is a particular risk in colder, wetter seasons where plant growth is slow (Kelly et al. 2006). Nitrate can reach surface waters through run-off, contaminate groundwater and impact on public health if the water is used as a potable resource and potentially cause eutrophication of groundwater-dependent ecosystems.

Australia has some of the oldest and least fertile soils in the world; therefore, the P in waste water is generally of great benefit to crops in Australia. Reclaimed wastewater typically con-

Table 2 Crop macronutrient uptake and supply in reclaimed water (kg/ha)

		Nutrient contents (kg/ha)				
Crop	Typical yield (t/ha)	N	P	K	Ca	Mg
Cabbage	50	147	24	147	36	13
Capsicum	20	41	4	69	52	7
Carrot	44	210	19	270	175	10
Cauliflower	50	181	28	225	127	18
Celery	190	308	97	700	290	38
Cucumber	18	66	12	120	34	8
Lettuce	50	100	18	180	10	3
Potato	40	264	23	310	66	21
Tomato	194	572	133	856	348	87
Reclaimed water[a]	1,000 mm	82	11.5	468	399	308

Source: Kelly et al. (2006)

[a] Nutrients applied in 1,000 mm from the Virginia Pipeline Scheme, South Australia

tains less than 3 mg/L of soluble P, which rapidly becomes adsorbed to soil particles after irrigation (Kelly et al. 2006). When plant demand is low, P accumulation and immobilisation in the soil is more likely than leaching or over-fertilisation; the exception is in sandy soils where there is some risk of leaching (Kelly et al. 2006). The main concerns associated with P are its potential toxicity to Australian natives which has evolved on our low P soils and run-off or accidental discharges to water bodies leading to eutrophication (NRMMCEP and HCAHMC 2006).

3.2 Heavy Metal(loid)s

Most heavy metal(loid)s are very effectively removed from wastewater in the treatment process so that their levels are very low in reclaimed water. Boron and Cd are the only two heavy metal(loid)s included in the list of key environmental hazards in the current Australian Guidelines for Water Recycling – they are not as readily separated from reclaimed water during standard treatment (NRMMCEP and HCAHMC 2006).

Metal(loid)s partition to the biosolids formed during sedimentation processes because their ionic nature causes them to sorb strongly to charged organic matter and clays (Unkovich et al. 2006). At low levels some heavy metal(loid)s are considered as micronutrients, but above plant requirements foliar application can produce phytotoxicity (Bolan et al. 2011). By virtue of their persistent nature, they can also accumulate in the soil, thereby resulting in soil biota toxicity, phytotoxicity through root uptake and entry into the food chain leading to negative impacts on food quality and human health.

Boron is not retained in biosolids because it exists as an uncharged species within the normal pH range of wastewater and thus remains in the reclaimed water. Boron is a micronutrient at very low levels; it has a narrow safety margin and, if leaching fractions are insufficient, it can accumulate in the soil profile and cause a reduction in yield and also phytotoxicity in sensitive species (Unkovich et al. 2006).

Cadmium presents the highest health risk of all the heavy metal(loid)s in reclaimed water; it is loosely bound to soil and will cause phytotoxicity at relatively low levels; it is a particular threat to humans and animals because toxicity occurs at a lower threshold than for plants. Consequently, there are national and international schemes to monitor the Cd concentration in foods (Unkovich et al. 2006).

3.3 Organic Contaminants

Three main groups of organic contaminants are found in reclaimed wastewater (GuangGuo 2006; Müller et al. 2007) that include (1) natural organic matter (NOM) which consists of refractory molecules like fulvic and humic acids; (2) disinfection by-products which are formed during chlorination and (3) synthetic organic compounds including pesticides, organohalothanes, phthalates, aromatic hydrocarbons, surfactants, endocrine disrupting chemicals (EDCs), pharmaceuticals and personal care products.

Although NOM can induce putrefaction in stored reclaimed water by depleting oxygen, there is little concern with discharging NOM onto agricultural land because it should eventually be broken down by the natural microbial populations (GuangGuo 2006). Disinfection by-products (DBP) can be formed by the reaction of chlorine with NOM (Singer 1999). Trihalomethanes are the most well known of the DBPs which are considered carcinogenic, mutagenic and clastogenic (Kim and Clevenger 2007). Synthetic organic compounds represent a wide range of chemicals. Some are susceptible to wastewater treatment processes whilst others fall into the group of stable organics which may remain in very small amounts in reclaimed water. Many have been implicated as EDCs which interfere with normal hormone communication systems; they impact adversely on growth, reproduction and development. There is limited data on their presence in wastewater, and due to their potential to cause adverse environmental and human health impacts, further monitoring and research are warranted (Holmes et al. 2010).

3.4 Salinity and Sodicity

Irrigating with reclaimed water carries a risk of inducing soil salinity and/or sodicity because reclaimed water often contains high levels of salts, in particular Na (Rengasamy 2006). Soil salinity is seen when an elevated concentration of soluble salts in the soil-water solution induces osmotic stress in vegetation. Sodicity is an increase in proportion of Na relative to the divalent cations and adversely affects soil structure.

Managing soil salinity has been identified as one of the largest threats to developing a sustainable recycled water scheme (Sumner and Naidu 1998; Stevens et al. 2003). The salts which contribute to salinity include Na^+, K^+, Ca^{2+}, Cl^-, Mg^{2+}, $SO_4{}^{2-}$ and $HCO_3{}^-$, but Na^+ and Cl^- ions exert the greatest environmental impact because their solubility in water renders them more available for interactions in the soil (NRMMCEP and HCAHMC 2006).

Reclaimed water can induce soil salinity when salts become concentrated in the soil through evaporation, the principle signs of which relate to osmotic stress. Salinity reduces plant growth because the increased osmolarity makes it difficult for plants to absorb water and nutrients. In response to reduced water uptake, plants produce the hormone abscisic acid which signals stomata to close, reducing transpiration water losses. Consequently, carbon dioxide absorption is reduced and photosynthesis slows leading to lower plant growth (Hartung et al. 1999).

To prevent salt accumulation, a leaching fraction must be incorporated into the crops irrigation requirements to drive salts below the root zone. However, given climatic variations in rainfall and evaporation throughout the year, supplying the correct leaching volume can be difficult. Maintaining soil structure so that the leaching fraction can permeate the soil layers is also critical and this is complicated further by sodicity.

Reclaimed waters frequently have higher levels of Na^+ ions compared to the other cations and can induce sodicity or saline sodicity (Bond 1998). Sodicity develops when free Na^+ ions bind to the cation exchange sites on clays and by this mechanism remain in the soil whilst the other free salts are leached downwards (Sumner and Naidu 1998; Rengasamy 2006). In situations where the other free salts remain, the soil is known as saline-sodic and the soil structure remains intact.

The extent to which Na^+ ions bind to the cation exchange sites on a clay particle is determined by the ratio of Na^+ ions to Ca^{2+} and Mg^{2+} ions in the soil solution. This can be expressed either as the percentage of Na which occupies the cation exchange capacity of a clay, the exchangeable sodium percentage (ESP), or the ratio of Na^+ ions to Ca^{2+} and Mg^{2+} ions in the soil solution, the sodium adsorption ratio (SAR).

Sodic soils have poor physical characteristics because the high levels of Na^+ interfere with the structural integrity of clay particles when the soil is wetted (Laurenson et al. 2010). As a consequence, sodic soils display the typical characteristics and problems that include (Rengasamy 2006) reduced porosity and permeability, reduced infiltration and hydraulic connectivity, surface crust formation which impedes infiltration and promotes run-off and erosion, difficult to cultivate, provide an impediment to the development of a root network and expose plant roots to anoxic or waterlogged conditions and slow plant growth.

Reduced drainage can also lead to further accumulation of salts through poor downward movement of irrigation water and evaporative concentration. Clays with low hydraulic conductivity are more prone to developing sodicity because they have a low leaching fraction (Rengasamy 2006), and these soils retain water in their profile which is subjected to evaporation, leaving salts behind.

4 Management Practices

4.1 Irrigation Methods and Management

When reclaimed water is used as an irrigation source, the crop irrigation requirement must be carefully calculated to avoid the effects of hydraulic loading which include waterlogging, poor crop growth and health, mobilisation of salts and contaminants, rising water tables and run-off (NRMMCEP and HCAHMC 2006). The irrigation requirement is essentially the difference between the crop water requirements and the rainfall but also must take into account the seasonal changes in rainfall, homogeneity of water infiltration and the leaching requirement (Christen et al. 2006). Whilst leaching is often necessary to drive salts below the root zone, it is important that it is not conducted at the expense of a rising water table. A balance must be reached between the total water requirements of the crop and preserving the normal hydrologic function. Regional and local groundwater levels should be monitored so that any changes can be detected and managed appropriately.

The pathogen content of reclaimed water is often the limiting factor with regards to irrigation method; for example, class A water can be applied to crops which are eaten raw by any irrigation method, water consistent with class B can only be used to irrigate these crops by furrow or dripper irrigation and class C must be applied by subsurface drippers. Furrow or flood irrigation has the advantage of being relatively inexpensive and low in manpower requirements, but unless it is well designed and managed, infiltration can vary greatly throughout the irrigation space. Because sprinklers commonly apply water directly on foliage, their use on produce consumed raw is limited to class A water. Even with class A water, direct ion toxicities (with saline reclaimed water) and an increased propensity to develop fungal disease can be a concern where foliar application of water occurs (Christen et al. 2006). The most efficient system with the least environmental and human risk is generally considered to be drip irrigation (Christen et al. 2006).

4.2 Best Practice Management

Best practice irrigation with reclaimed water cannot be achieved by a one-solution-fits-all approach because there are a multitude of variables which must be considered, and each enterprise is unique. Irrigation schemes using reclaimed water should be tailored to optimise the economic returns to the grower whilst also minimising the impact on the receiving environment. This can best be achieved by undertaking a comprehensive risk assessment of the whole scheme and designing an irrigation management plan to minimise the risk of adverse outcomes.

The risk assessment should be based on the potential health impacts and soil, site and wastewater characteristics as follows:

- The soil properties examined should include soil texture, topsoil depth, depth to drainage or root impeding layers, infiltration rates, soil-water holding capacity and soil chemistry.
- Site characterisation must make assessment of topography, slope, soil homogeneity, history of waste storage or disposal on site, depth to groundwater and seasonal or permanent water tables, areas of drainage hazard and separation distances from sensitive areas.
- Wastewater analysis should describe the reclaimed water with reference to total solids, suspended and volatile solids, total P, inorganic P, total N, NH_4^+-N, K, SO_4^{2-}, BOD, pH, electrical conductivity, SAR, Ca, Mg, organic C, Na and Zn.
- Potential health impacts must be addressed with the relevant state health authority.

5 Case Study: Northern Adelaide Plains Irrigation Scheme

The Northern Adelaide Plains Reclaimed Water Scheme (or Virginia Pipeline Scheme), South Australia, provides irrigation for over 20 different types of crops within an area of approximately 200 km^2 (Laurenson et al. 2010). It was the first scheme of its type in Australia and remains as one of the largest reclaimed water schemes in the southern hemisphere. It supplies approximately 180 GL of tertiary treated, class A wastewater from the Bolivar Wastewater Treatment Plant (WWTP) to horticultural growers on the Northern Adelaide Plains through more than 100 km of pipelines (Laurenson et al. 2010). In 2008, the scheme encompassed 400 connections with the capability to supply up to 105 ML/day during the peak seasons; it delivers nearly half the water required by growers at Virginia. The water is used to irrigate a wide range of fruit and vegetables which supply local and interstate markets including beans, broccoli, cabbage, capsicum, carrots, cucumber, eggplants, lettuce, melons, onions, parsnips, pears, potatoes, pumpkins, tomatoes, zucchini, nuts, olives and wine grapes (Marks and Boon 2005).

The scheme is a joint venture between the Virginia Irrigators Association (representing the growers), Water SA (the state water authority responsible for wastewater treatment) and a private company, Water Infrastructure Group, Tyco. The establishment of this scheme in 1999 was largely driven by local growers facing a shortage of irrigation water.

The Virginia Pipeline Scheme has provided a secure water resource during a period which has been one of the driest on record. In some cases, the reclaimed water has replaced groundwater resources and in others provided a water source where farmers were unable to receive a groundwater allocation. The scheme has ensured the long-term economic sustainability of Adelaide's food bowl. The recycled water is sold at a reduced rate compared to mains water. About $50 million worth of the produce grown in the area each year uses the reclaimed water. The Water Infrastructure Group translates this to a $1 billion benefit to the district over the first 10 years of the project.

Environmentally, the scheme results in 35 % of water being recycled at Bolivar WWTP, reduces the discharge of harmful nutrients into the marine environment, reduces demand for groundwater extractions and contributes to reducing South Australia's dependence on pressured surface water systems. As one proponent eloquently summed it up, "The scheme has operated for 10 years with no human health issues and no detrimental environmental impacts, proving that recycled water can provide a safe and sustainable water resource."

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A Review of Biopesticides and Their Mode of Action Against Insect Pests

Sengottayan Senthil-Nathan

Abstract

Biopesticides, including entomopathogenic viruses, bacteria, fungi, nematodes, and plant secondary metabolites, are gaining increasing importance as they are alternatives to chemical pesticides and are a major component of many pest control programs. The virulence of various biopesticides such as nuclear polyhedrosis virus (NPV), bacteria, and plant product were tested under laboratory conditions very successfully and the selected ones were also evaluated under field conditions with major success. Biopesticide products (including beneficial insects) are now available commercially for the control of pest and diseases. The overall aim of biopesticide research is to make these biopesticide products available at farm level at an affordable price, and this would become a possible tool in the integrated pest management strategy. Moreover, biopesticide research is still going on and further research is needed in many aspects including bioformulation and areas such as commercialization. There has been a substantial renewal of commercial interest in biopesticides as demonstrated by the considerable number of agreements between pesticide companies and bioproduct companies which allow the development of effective biopesticides in the market. This paper has reviewed the important and basic defection of major biopesticides in the past. The future prospects for the development of new biopesticides are also discussed.

1 Introduction

1.1 Biopesticides

Biopesticides are developed from naturally occurring living organisms such as animals, plants, and microorganisms (e.g., bacteria, fungi, and viruses) that can control serious plant-damaging insect pests by their nontoxic eco-friendly mode of

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actions, therefore reaching importance all over the world. Biopesticides and their by-products are mainly utilized for the management of pests injurious to plants (Mazid et al. 2011).

Biopesticides are classified into three different categories: (1) plant-incorporated protectants, (2) microbial pesticides, and (3) biochemical pesticides. They do not have any residue problem, which is a matter of substantial concern for consumers, specifically for edible fruits and vegetables. When they are used as a constituent of insect pest management, the efficacy of biopesticides can be equal to that of conventional pesticides, particularly for crops like fruits, vegetables, nuts, and flowers. By combining synthetic pesticide performance and environmental safety, biopesticides execute efficaciously with the tractability of minimum application limitations and with superior resistance management potential (Kumar 2012; Senthil-Nathan 2013).

Copping and Menn (2000) reported that biopesticides have been gaining attention and interest among those concerned with developing environmentally friendly and safe integrated crop management (ICM)-compatible approaches and tactics for pest management. In particular, farmers' adoption of biopesticides may follow the recent trend of "organically produced food" and the more effective introduction of "biologically based products" with a wide spectrum of biological activities against key target organisms, as well as the developing recognition that these agents can be utilized to replace synthetic chemical pesticides (Menn and Hall 1999; Copping and Menn 2000; Chandrasekaran et al. 2012; Senthil-Nathan 2013).

Insecticides from microorganisms extend a unique chance to developing countries to research, and they have possessed to develop natural biopesticide resources in protecting crops. The utilization of biopesticide programs would be required to prevent the development of resistance in target insect pests to synthetic chemical pesticides and toxins from biopesticides (Copping and Menn 2000; Senthil-Nathan 2006; Senthil-Nathan et al. 2006, 2009).

Compared with chemical pesticides, biopesticides do not present the same regulatory problems seen with chemical pesticides. Biopesticides are frequently target specific, are benign to beneficial insects, and do not cause air and water quality problems in the environment, and also agricultural crops can be reentered soon after treatment. Microorganisms from nature can also be used in organic production, and risks to human health are low. In addition, the usage of biopesticides has other several advantages; e.g., many target pests are not resistant to their effects (Goettel et al. 2001; EPA 2006).

Biopesticides derived from bacteria like *Bacillus thuringiensis* (Bt), a large array of fungi, viruses, protozoa, and some beneficial nematodes have been formulated for greenhouse, turf, field crop, orchard, and garden use (Hom 1996; Butt et al. 2001a, b; Grewal et al. 2005; EPA 2006). Biocontrol microbials, their insecticidal metabolic products, and other pesticides based on living organisms are sorted as biopesticides by the EPA. There are hundreds of registered products enlisted in EPA (2013).

2 Microbial Pesticides

2.1 Bacteria

2.1.1 *Bacillus thuringiensis*

Beginning in the 1980s and continuing to the present, a different molecular approach has been employed to develop market acceptance of biopesticides. Earlier, several efforts were aimed at establishing microbial insecticides, like *Bt*, which has been used commercially over 40 years (Gelernter and Schwab 1993). Later, some *Bacillus* species such as *Bacillus thuringiensis israelensis Bti* and *Bacillus sphaericus* 2362 (*Bs*) were found particularly effective against mosquito (Revathi et al. 2013) and other dipteran larvae. *Bti* was first discovered to have increased toxicity against mosquito larvae in 1975 (Goldberg and Margalit 1977).

Various bacterial species and subspecies, especially *Bacillus*, *Pseudomonas*, etc., have been established as biopesticides and are primarily used to control insect and plant diseases. Most salient among these are insecticides based on

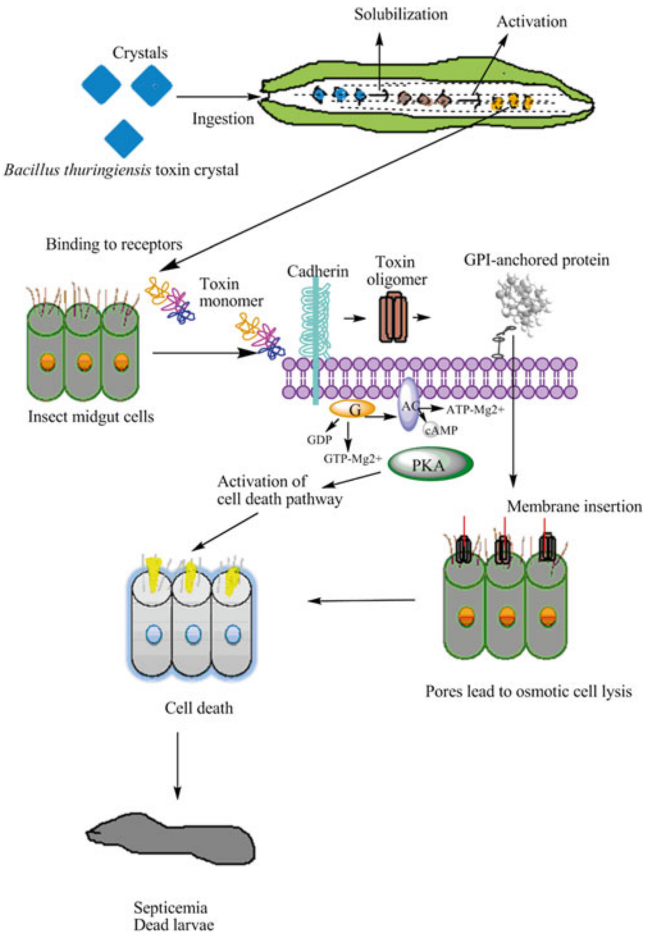

Fig. 1 Mode of action of *Bt* toxin against lepidopteran insects

several subspecies of *Bacillus thuringiensis* Berliner. These include *B. thuringiensis* ssp. *kurstaki* and *aizawai*, with the highest activity against lepidopteran larval species; *B. thuringiensis israelensis*, with activity against mosquito larvae, black fly (simuliid), and fungus gnats; *B. thuringiensis tenebrionis*, with activity against coleopteran adults and larvae, most notably the Colorado potato beetle (*Leptinotarsa decemlineata*); and *B. thuringiensis japonensis* strain Buibui, with activity against soil-inhabiting beetles (Carlton 1993; Copping and Menn 2000).

Bt produces crystalline proteins and kills few target insect pest species like lepidopteran species. The binding of the *Bt* crystalline proteins to insect gut receptor determines the target insect pest (Kumar 2012).

Toxicity of *Bti* and some other toxic strains is commonly imputed to the parasporal inclusion bodies (δ-endotoxins) which are produced during sporulation time. These endotoxins must be assimilated by the larvae to accomplish toxicity. *Bt* and their subspecies produce different insecticidal crystal proteins (δ-endotoxins), and their toxicity was determined (Chilcott et al. 1983; Aronson and Shai 2001). These toxins, when ingested by the larvae, can damage the gut tissues, leading to gut paralysis. After that, the infected larvae stop feeding and finally they die from the combined effects of starvation and midgut epithelium impairment (Fig. 1) (Betz et al. 2000; Zhu et al. 2000; Darboux et al. 2001).

Some other microbial pesticides act by outcompeting insect pest organisms. Microbial pesticides need to be continuously supervised to ensure that they do not become capable of injuring nontarget organisms, including humans (Mazid et al. 2011). In previous studies, the microbial pesticide advance has resulted in a significant decrease of synthetic chemical insecticide usage (James 2009).

Gray et al. (2006) reported *Bt* toxins produced by plant growth-promoting rhizobacteria, which also develop bacteriocin compounds of insecticidal attributes. *Bt* is marketed worldwide for the control of different important plant pests, mainly caterpillars, mosquito larvae, and black flies. Commercial *Bt*-based products include powders containing a combination of dried spores and crystal toxins. They are applied on leaves or other environments where the insect larvae feed. Toxin genes from *Bt* have been genetically engineered into several crops.

3 Fungi

3.1 *Metarhizium anisopliae*

M. anisopliae Sorokin var. *anisopliae* is an essential entomopathogenic fungus. It propagates worldwide in the soil, demonstrating a wide range of insect host species. This subspecies was first described in 1879 by Metschnikoff, under the term *Entomophthora anisopliae*, as a pathogen of the wheat cockchafer, and later it was termed *M. anisopliae* by Sorokin in 1883 (Tulloch 1976).

Several entomopathogenic fungi and their derivatives are also used as microbial pesticides. *M. anisopliae* are hyphomycete entomopathogenic fungi most widely used for insect pest control and are ubiquitous worldwide. This species comprises a huge number of different strains and isolates of various geographical origins and from different types of hosts (Roberts and St. Leger 2004).

Under natural conditions, *Metarhizium* are found in the soil, where the moist conditions permit filamentous growth and production of infectious spores, called conidia, which infect soil-dwelling insects upon contact (Fig. 2). *M. anisopliae* has the potential to be used as a biocontrol agent, particularly for malaria vector species, and is also a suitable candidate for further research and development (Mnyone et al. 2010).

Driver et al. (2000) reevaluated the taxonomy of the genus *Metarhizium* using sequence data from ITS and 28S rDNA D3 regions and also using RAPD patterns, revealing ten distinguishable clades. *M. anisopliae* var. *anisopliae* represents clade 9. These entomopathogenic fungi have been viewed as safe and regarded as an environmentally satisfactory alternative to synthetic chemical pesticides (Domsch et al. 1980; Zimmermann 1993). Recently, these entomopathogenic fungi have been registered as microbial agents and are also under commercial development for the biological control of several pests (Butt et al. 2001a, b).

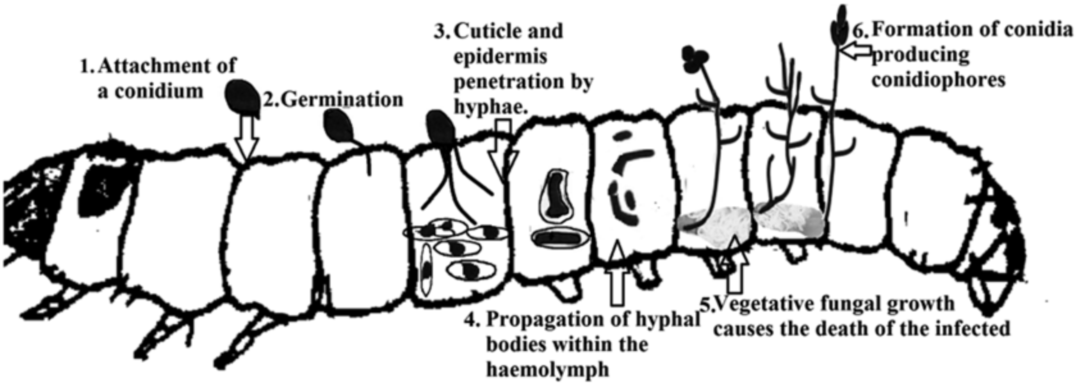

Fig. 2 Mode of action of entomopathogenic fungi against lepidopteran insects

M. anisopliae strains are obtained from different geographical localities (Fegan et al. 1993), as suggested, among others, by extremely variable toxicities (Goettel and Jaronski 1997). *M. anisopliae* have been used on a large scale in countries like Brazil, where 100,000 ha of sugarcanes are treated every year (Faria and Magalhães 2001). They are released in the field after thorough assessment of strains. Consorting to Goettel et al. (2001) have reported that the growth and application of fungi as microbial agents for biocontrol of insect pest involve tests with mammalian models to evaluate possible human and animal health risks.

4 Virus

4.1 Baculovirus

Baculoviruses are double-stranded DNA viruses present in arthropods, mainly insects. Baculoviruses are usually highly pathogenic and have been used efficaciously in their natural form as biocontrol agents against numerous serious insect pests (Moscardi 1999). Even so, the application of baculoviruses is still limited in the field of agriculture and horticulture where the thresholds for pest damage tend to be minimized. In Lepidoptera, i.e., the primary group where baculoviruses have been isolated, they only cause mortality in the larval stage (Cory 2000).

Baculoviruses need to be ingested by the larvae to initiate infection. After ingestion, they enter the insect's body through the midgut and from there they spread throughout the body, although in some insects, infection can be limited to the insect midgut or the fat body (Fig. 3). Two groups of baculoviruses exist: the nucleopolyhedroviruses (NPVs) and granuloviruses (GVs). In NPVs, occlusion bodies comprise numerous virus particles, but in GVs, occlusion bodies ordinarily contain just one virus particle. A common feature of baculoviruses is that they are occluded, i.e., the virus particles are embedded in a protein matrix. The presence of occlusion bodies plays an essential role in baculovirus biology as it allows the virus to survive outside the host (Cory 2000).

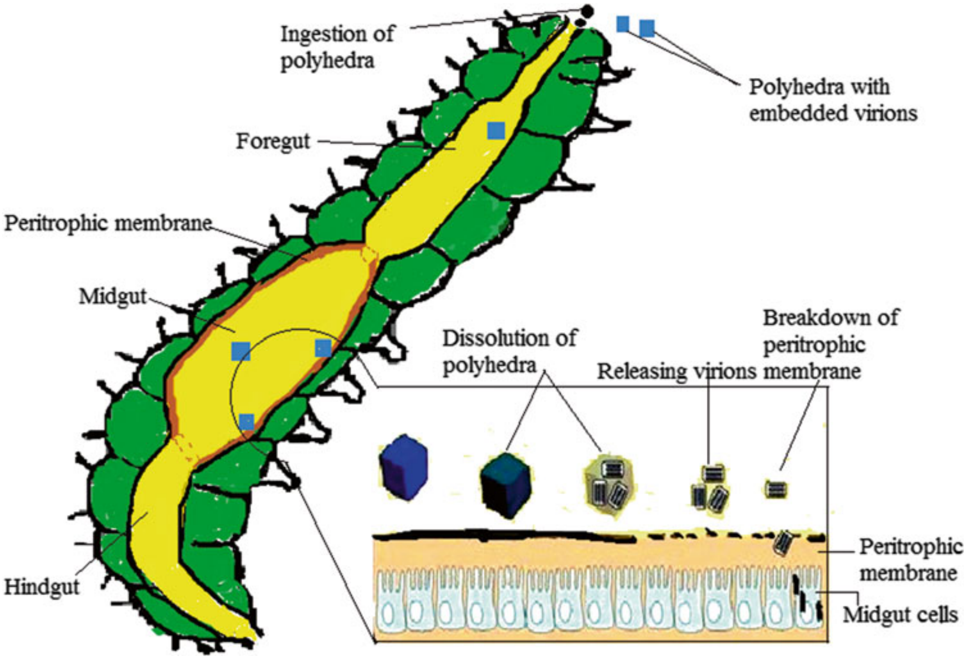

Fig. 3 Mode of action of baculoviruses against lepidopteran insects

Recently, it has been substantially demonstrated that baculoviruses are not infectious to vertebrates and plants. Also within the insects, their host range is restricted to the order from which they were isolated. Genetic engineering of baculoviruses has so far been restricted to isolates from Lepidoptera (Kolodny-Hirsch et al. 1997). Baculoviruses vary in the number of hosts that they can infect; some seem to be particular to one species and hence are very unlikely to be a hazard when genetically modified, whereas others infect a range of hosts (Barber et al. 1993; Richards et al. 1999; Cory et al. 2000).

The majority of new baculovirus recombinants now employ insect-selective toxins. Genetic modification has been mostly carried out on the alfalfa looper, *Autographa californica* NPV (AcNPV)-the virus for which most molecular information is available, including the complete DNA sequence, which permits precise insertion of foreign genes. Recently, the development of genetically modified baculoviruses has expanded to other strains of commercial or regional interest (Popham et al. 1997; Cory 2000).

Baculovirus-infected insect larvae have primarily initiated a cascade of molecular and cellular appendages finally the larva enters into the death and the development of huge amounts of polyhedral occlusion bodies comprising rod-shaped virions (Miller 1997). Lately, a naturally occurring baculovirus (*Agrotis ipsilon* multiple nucleopolyhedrovirus, family Baculoviridae, AgipMNPV) was indicated to have hope as a microbial insecticide for controlling *A. ipsilon* in turf (Prater et al. 2006).

Numerous viral formulations are available primarily for the control of caterpillar pests. For instance, Certis has recently registered Madex™, an increased-potency codling moth granulosis virus (GV) that also affects oriental fruit moth (OFM). Certis also deals Cyd-X™, which also contains the codling moth GV and which can be an efficient tool for codling moth management (Arthurs and Lacey 2004; Arthurs et al. 2005). Aside from Madex and Cyd-X, Certis markets Gemstar™, which contains *Heliothis zea* NPV, and Spod-X™, which contains beet armyworm NPV. Gemstar is also registered for the control of lepidopteran pests, like the cotton bollworm and budworm, caterpillars that are mainly dangerous insect pests of corn, soybean, and other vegetables (Arthurs et al. 2005). Furthermore, Certis has registered a celery looper (*Syngrapha falcifera*) NPV and an alfalfa looper (*Autographa californica*) NPV (EPA 2006).

5 Nematodes

5.1 *Steinernema* (Rhabditida)

One of the new hot products in biopesticide is nematodes. Pest nematodes can be supervised with cover crops, crop rotation, and internalization of organic material into the soil (McSorely 1999). In the early 1990s, various effective entomopathogenic nematodes from two genera, namely, *Steinernema* and *Heterorhabditis* (Nematoda: Rhabditida), were discovered and established as a biocontrol agent against insects (Copping and Menn 2000).

Insect-parasitic nematodes may encroach upon soil-dwelling stages of insects and kill them within 48 h through the expulsion of pathogenic bacteria. After the host dies, the infectious stages of the nematodes become adults and a modern generation of infective juveniles (IJs) develops (Fig. 4). Entomopathogenic nematodes are commonly available for plant protection from serious insect pests and diseases, and also there have been various efforts to biocontrol insect pest populations in the field by employing IJs via spraying. Nevertheless, little is known about the ability of indigenous nematodes to influence insect pest populations (Peters 1996).

In nematodes, the parasitic cycle is initiated by the third-stage IJs. These nonfeeding juveniles infest suitable insect host and enter through the insect's natural body openings like the anus, mouth, and spiracles (Grewal et al. 1997). Once they have entered inside the host, nematodes infest the hemocoel and then release their symbiotic bacteria into the intestine. After that, the bacteria cause septicemia, killing the host within 24–48 h (Fig. 4). The uptake of IJs is rapidly manipulating by the bacteria and decomposed the

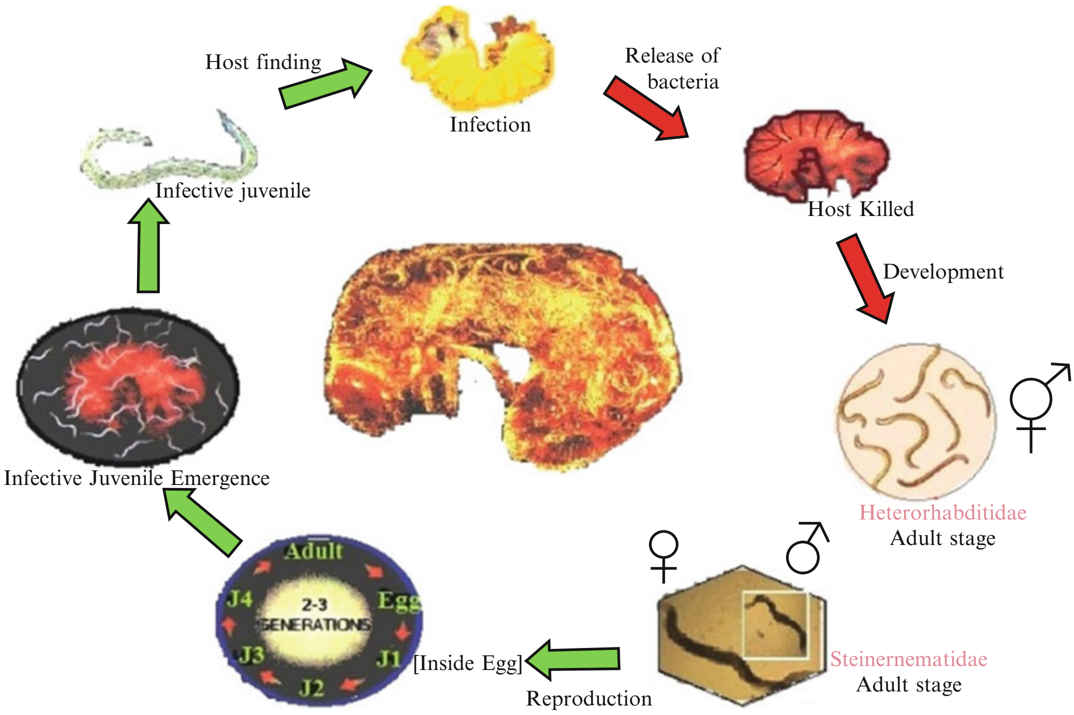

Fig. 4 Mode of action of entomopathogenic nematodes against lepidopteran insects

host tissues. Almost two to three generations of the nematodes are finished within the host cadaver (Pomar and Leutenegger 1968; Bird and Akhurst 1983).

In previous research studies, it has been clearly indicated that entomopathogenic nematodes can be effective biocontrol agents against some of the major insect pest families found in stored goods like Pyralidae (Shannag and Capinera 2000) and Curculionidae (Duncan and McCoy 1996; Shapiro and McCoy 2000). Earlier, Morris (1985) had demonstrated the susceptibility of insect pests found in stored products, including *Ephestia kuehniella* Zeller and *Tenebrio molitor* L., to an increased concentration of nematodes. Georgis (1990) had also advocated a field concentration of >2.5 billion nematodes/ha against some of the major insect pests of row crops, but concentrations few times higher (7–15 billion/ha) are demanded to accomplish the control of pest population (Loya and Hower 2002).

6 Protozoa

6.1 *Nosema*

In previous studies, protozoan diseases of insects are ubiquitous in nature and constitute an essential regulatory role in insect populations (Maddox 1987; Brooks 1988). Microsporidia, such as *Nosema* spp., are generally host specific and slow acting, most frequently producing chronic infections. The biological activities of most entomopathogenic protozoa are complex. They grow only in living hosts and some species necessitate an intermediate host. Microsporidia species are among the most commonly observed, and their main benefits are persistence and recycling in host populations and their debilitating effect on reproduction and overall fitness of target insects. As inundatively utilized microbial control agents, some species have been moderately successful (Solter and Becnel 2000).

Some protozoan species are pathogenic like *Nosema locustae* for grasshoppers, which is the only species that has been registered and established commercially (Henry and Oma 1981). *N. bombycis*, the first reported microsporidium, is a pathogen of silkworm pébrine, which persisted in Europe, North America, and Asia during the mid-nineteenth century (Becnel and Andreadis 1999). Pébrine is still an epidemic and causes heavy economic losses in silk-producing countries such as China (Cai et al. 2012).

Almost 1,000 protozoan species, mainly microsporidia, attack invertebrates, including numerous insect species like grasshoppers and heliothine moths. Virtually renowned insect-pathogenic protozoan species are *Nosema* spp. and *Vairimorpha necatrix*. Protozoans produce spores, which are the infectious phase in several susceptible insects. *Nosema* spp. spores are assimilated by the host and develop in the midgut. Germinating spores are released from the sporoplasm and invade host target cells, inducing massive infection and demolishing organs and tissues. Sporulation process begins again from the infected tissues and, upon expulsion and ingestion by a susceptible host, induces an epizootic infection. Naturally, parasitoids and insect predators commonly act as vectors distributing the disease (Brooks 1988).

7 Botanical Insecticides

Since ancient times, natural compounds from plants were used, more or less efficaciously to give security from insect pests. In the nineteenth century, these compounds became scientifically established and widely utilized in the earlier period of the twentieth century (Morgan 2004). Plants and some insects have coexisted on the earth for almost three and a half million years, which has allowed lots of time for both to develop offensive and defensive strategies. Plants have developed many strategies to assist themselves from being assaulted by predators. An example of such plant strategy is developing compounds that are highly toxic to insects (Warthen and Morgan 1985; Arnason et al. 1989; Morgan and Wilson 1999; Nisha et al. 2012).

7.1 Neem

In Asia, neem has an extensive history of use mainly against household and storage pests and, to some extent, against insect pests of crops. Nevertheless, a breakthrough in the insecticidal application of neem was attained by Pradhan et al. (1962) who successfully protected the crops from insects by applying them with low concentration of 0.1 % neem seed kernel suspension during a locust invasion. The Indian neem tree (*Azadirachta indica*) is one of the most important limonoid-producing plants from the Meliaceae family. Several components of its leaves and seeds show marked insect control potential, and due to their relative selectivity, neem products can be recommended for many programs on crop pest management (Schmutterer 1990). Neem product activity has been assessed against 450–500 insect pest species in different countries around the world, and from that, 413 insect pest species are reportedly susceptible at various concentrations (Schmutterer and Singh 1995).

In India alone, neem activity has been assessed against 103 species of insect pests, 12 nematodes, and several pathogenic fungi (Singh and Kataria 1991; Arora and Dhaliwal 1994). Some recent reviews on the potential of neem in pest management include those of Singh (1996, 2000), Singh and Raheja (1996), Naqvi (1996), Saxena (1998), and Dhaliwal and Arora (2001). Most works have focused on azadirachtin (Fig. 5) richly from neem seed extracts which act as both strong antifeedants and insect growth regulators. Azadirachtin affects the physiological activities of insects (Mordue (Luntz) and Blackwell 1993) and does not affect other biocontrol agents. Further, neem products are biodegradable and nontoxic to nontarget organisms (Senthil-Nathan 2013).

In several Asian countries, numerous studies have measured neem activity alone or in combination with established insecticides and other biocontrol agents of damaging insect pests in agricultural crop system (Abdul Kareem et al. 1987; Senthil-Nathan et al. 2005a, 2006). In Indian field trials carried out, neem treatments were determined to be effective against some insect species like green leafhopper, yellow stem

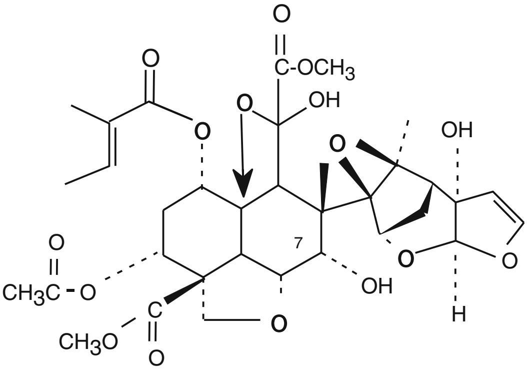

Fig. 5 Structure of azadirachtin

borer, rice gall midge, rice leaffolder, and grasshopper (Dhaliwal et al. 1996; Nanda et al. 1996; Senthil-Nathan et al. 2009).

7.2 *Melia azedarach*

The promotion of botanicals as eco-friendly pesticides, microbial sprays, and insect growth regulators has been a major concern amid the presence of other control measures like beneficial insects, all of which demand an integration of supervised insect pest control (Ascher et al. 1995). Plant-based insecticides are developed naturally from plant chemicals extracted for use against serious insect pests. As a result of concerns about the ecological continuity of synthetic pesticides and their potential toxicity to humans, nontarget beneficial insects, and some domestic animals, there is a regenerated interest in natural products to control insect pests. From this conclusion, the development of biopesticides seems to be a logical choice for further investigation. Meliaceae and Rutaceae species have received much attention due to the fact that they are a rich source of triterpenes known as limonoids (Connolly 1983).

The Meliaceae plant family is known to hold an assortment of compounds with insecticidal, antifeedant, growth-regulating, insect-deforming, and growth-modifying properties (Champagne et al. 1989; Schmutterer 1990; Mordue (Luntz) and Blackwell 1993; Senthil-Nathan and Kalaivani 2005, 2006; Senthil-Nathan 2006; Senthil-Nathan et al. 2004, 2005a, b, c).

M. azedarach, otherwise known as chinaberry or Persian lilac tree, is a deciduous tree that originates from northwestern India, and it has been recognized for its insecticidal properties, which are still to be entirely analyzed. This tree grows in the tropical and subtropical parts of Asia, but nowadays it is also cultivated in other warm places of the world because of its considerable climatic tolerance. The leaves of *M. azedarach* are used for their insecticidal activity, whereas the fruit extracts of *M. azedarach* produce a variety of effects in insects, such as growth retardation, reduced fecundity, molting disorders, and behavior changes (Ascher et al. 1995).

The antifeedant and insect growth-regulating effects of *M. azedarach* extracts are known for many insects (Connolly 1983; Saxena et al. 1984; Champagne et al. 1992; Schmidt et al. 1998; Juan et al. 2000; Carpinella et al. 2003; Senthil-Nathan 2006; Senthil-Nathan and Sehoon 2006), the latter effect being the most essential physiological effect of *M. azedarach* on insects (Ascher et al. 1995).

As previously mentioned, the Meliaceae plant family has been known as a potential source for insecticide properties. Also, several extracts from neem and other plant seeds and leaves have excellent insecticidal properties against vectors and are at the same time very eco-friendly (Schmutterer 1990; Senthil-Nathan et al. 2005a, b, c). The efficaciousness of these neem products on mosquitoes was also demonstrated (Chavan 1984; Zebitz 1984, 1986; Schmutterer 1990; Su and Mulla 1999; Senthil-Nathan et al. 2005d).

Without a doubt, plant-derived toxicants are a valuable source of potential insecticides. Plants and other natural insecticides may play a vital role in mosquito control programs as well as in other major insect control programs (Mordue (Luntz) and Blackwell 1993).

8 Biochemical Pesticides

8.1 Pheromones

Insects produce chemicals called pheromones to stimulate a certain behavioral reaction from other individuals. These pheromones have numerous effects and are named according to their evoked response, for example, sex pheromones, aggregation pheromones, alarm pheromones, etc. A few pheromones function as sex attractants, permitting individuals to detect and locate mates, whereas others induce trail following, oviposition, and aggregation in other congeners. Pheromones have become essential tools for monitoring and controlling agricultural pest populations, and as such, a huge collection of over 1,600 pheromones and sex attractants has been reported (Witzgall et al. 2004).

In the past decades, there has been a significant volume of literature on insect pheromones and new opportunities have arisen to explore the use of semiochemicals in managing insect pest problems. Insect control by pheromones alone has precincts, but they can be applied in integrated control in combination with other practices (Howse et al. 1998; Reddy and Guerrero 2004). Plant volatiles are recognized as an integral part of the pheromone system of various Coleopteran species studied so far. How the combinations of pheromones and plant volatiles are incorporated into the insects' olfactory systems so that they can discriminate pheromone molecules alone and pheromone plus odor plume strands and react behaviorally to these signals is a question of increasing importance (Baker and Heath 2004).

Recently, the pest management system has undergone an intentional shift from calendar-based, broad-spectrum insecticide applications to more holistic, integrated, and high-efficacy approaches. Furthermore, environmental conservation, food safety, and resistance management are a few of the key components guiding current pest management policies in commercial agriculture (Witzgall et al. 2010).

Kogan and Jepson (2007) had reported that meeting the necessities of the quickly expanding global population while incorporating sustainability and ecological stewardship is a major challenge facing modern agriculture. In agricultural fields, the application of pheromones and/or allelochemicals as behavioral manipulation tools can replace or complement existing pest management programs (Witzgall et al. 2008), resulting in a decreased rate of broad-spectrum use.

Nowadays, pheromones and other semiochemicals are applied to monitor and control pests in millions of hectares. There are several advantages of utilizing pheromones for monitoring pests, including lower costs, specificity, ease of use, and high sensitivity (Wall 1990; Laurent and Frérot 2007; Witzgall et al. 2010). Insect pest monitoring by using pheromone lures can profit management conclusions such as insecticide application timing (Leskey et al. 2012; Peng et al. 2012).

Pheromones produced by insects are highly species specific. Virgin female insects are developing sex pheromones when expecting for a mate and males along the concentration slope for the female producer. Aggregation pheromones are released by insects such as wood-invading beetles to show to others the presence of a good food source (Copping and Menn 2000).

Some of the alarm pheromones are developed by insects that are beneath approach from a predator and this contributes to a movement of the insect pest aside from the production source and, therefore it becomes dangerous. Plants and its derived attractants are also known that interact with the insects to a valuable food source and, while combine with insect-derived attractants will be developed a potent attraction to some insect pests (Copping and Menn 2000).

In previous studies, Mayer and McLaughlin (1991) proposed that all insects produce approximate form of pheromone and companies subsist that synthesize a pheromone for any customer. Recently, 30 mating-disruption pheromone-based products are registered by the US EPA as biocontrol agents of lepidopteran pest species that can cause agricultural damage (Copping and Menn 2000).

9 Conclusion

The utilization of natural products with commercial value is directly manifested by the numerous compounds present in the market and that have remained there in many cases after many years. These values of natural products are considered as a source of new mechanisms and their consequent incorporation into high-output screens is hard to evaluate. Recently, several works have been exercised and extend to be undertaken to enhance the shelf life, immediate death, the biological scheme, efficient in the field and dependability, and the effect of cost of living systems and there have been some notable successes in situations where some disruption to the crop is acceptable.

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Seaweeds: A Promising Source for Sustainable Development

T. Nedumaran and D. Arulbalachandran

Abstract

Macroscopic marine algae, popularly known as seaweeds, form one of the important living resources of the ocean. Seaweeds form an important renewable resource in the marine environment and have been a part of human civilization from time immemorial. Reports on the uses of seaweeds have been cited as early as 2,500 years ago. Seaweed utilization for a variety of purposes has led to the gradual realization that some of their constituents are more superior and valuable in comparison to their counterparts on land. Seaweeds synthesize a wide range of chemicals, some of which are the only natural resources of agar, carrageenan, and alginate. These have been used as food for human beings, feed for animals, natural biofertilizers for plants and source of various chemicals like pharmaceutical values without any side effects, production of biodiesel and wastewater management, etc. In this chapter, utilization of seaweeds has been obviously discussed which are proving and playing a crucial role in sustainable development to became a promising source for human welfare.

Keywords

Biofertilizers • Biofuels • Animal feed • Pharmaceutical value

1 Introduction

Seaweeds or benthic marine algae are the group of plants that live either in marine or brackish water environment. Like the terrestrial plants, seaweeds contain photosynthetic pigments, and with the help of sunlight and nutrient present in

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the seawater, they photosynthesize and produce food. Seaweeds are found in the coastal region between high tide and low tide and in the subtidal region up to a depth where 0.01 % photosynthetic light is available. Plant pigments, light, exposure, depth, temperature, tides, and the shore characteristics combine to create different environments that determine the distribution and variety among seaweeds (Dhargalkar and Kavlekar 2004). Seaweeds or marine macroalgae are primitive nonflowering plants, without true root, stem, and leaves. They form one of the commercially important marine living renewable resources. Seaweeds occur in the intertidal shallow and deep waters of the sea and also in estuaries and backwaters. They grow on rocks, dead corals, stones, pebbles, solid substrata, and other plants. They require certain environmental conditions for proper growth and establishment in different regions of the coastline. However, the topography, physical nature of the substratum, salinity, currents, tidal action, and other factors of the marine environment vary in different parts of the coastline, and as a result of these fluctuations, marked changes occur in the distribution and abundance of different kinds of seaweeds.

1.1 Seaweed Wealth in India

India has a vast coastline more than 9,000 km long, a number of estuaries, backwaters, and island, rocky, or coral formations occurring in Tamil Nadu and Gujarat states and in the vicinity of Bombay, Karwar, Ratnagiri, Goa, Vizhinjam, Varkala, Visakhapatnam, and few other places like Chilka and Pulicat lakes. India is rich in algal biodiversity country and has large stretches of suitable area for growth. According to Oza and Zaidi (2001), totally 844 species of marine algae available in India belong to different genera and classes as shown below:

	Genera	Species
Chlorophyceae	43	216
Phaeophyceae	38	194
Rhodophyceae	136	434
	217	844

2 Classification of Seaweeds

Based on the type of pigments, external and internal structure, and reproduction, seaweeds are divided in to four broad groups: green, blue-green, brown, and red. Botanist refers to these broad groups as Phaeophyceae, Rhodophyceae, Cyanophyceae, and Chlorophyceae, respectively. Brown seaweeds are usually large and range from the giant kelp; red seaweeds are usually smaller, generally ranging a few centimeters to about a meter in length and they are not always red. They exhibited sometimes purple and even brownish red. Green seaweeds are also small with a similar size range to the red seaweeds.

3 Ecology and Biology of Seaweeds

Seaweeds are ecologically important primary producers, competitors, and ecosystem engineers that play a central role in coastal habitats ranging from kelp forests to coral reefs. Although seaweeds are known to be vulnerable to physical and chemical changes in the marine environment, the impacts of ongoing and future anthropogenic climate change in seaweed-dominated ecosystems remain poorly understood (Harley et al. 2012). Ecological studies have been carried out on the marine algal vegetation of different localities of Indian coast by various workers. They provided data on the seasonal changes and zonation of algae and on the environmental conditions existing in those areas. The changes in the total emergence and submergence, topography of the coast, surf action, and levels at which the plants grow were found to contribute much to the variation in the growth of the algae. Every year fresh plants develop from the reproductive bodies liberated by the plants of the previous generation or from the perennial basal portion of the old plants. The period of regeneration and increase and decline in growth vary from species to species and also from one locality to the other. In some seaweeds, two peak growth periods with a half-yearly growth cycle were observed, while in other seaweeds, only a single peak growth period was observed. In general, maximum growth has been observed in many algae in two seasons of the year, one from June to August and another from November to January. The commercially useful algae should be collected only during their peak growth periods in order to get more quantity of raw material and better yield of finished products with good quality.

Studies on the fruiting periods and relative preponderance of vegetative and reproductive phases of many commercially important seaweeds indicated that the fruiting behavior varies in different seaweeds growing along the Indian coast. Though reproduction was observed throughout the year, two fruiting seasons in a year in many algae and one fruiting season in other algae were found. Information on the spore output from economically important algae was collected. As enormous number of spores was found to be produced from an alga, they can be successfully raised to germlings in the laboratory or nursery and then to harvestable size plants in the sea by transplantation. Periodicity or rhythm in the liberation of spores was observed in some seaweeds, while there was no such periodicity in the shedding of spores in other seaweeds. The spore output season and also the period of maximum sporulation varied from species to species. Information was collected also on the spore germination, survival of germlings, and life cycle of some marine algae. The results of several studies on the growth, fruiting behavior, and sporulation of the economically important marine algae growing in different area paved the way to utilize the available marine algal resources in a rational way for commercial exploitation and cultivation. Studies on the diversity, physiology, biochemistry, and utilization of potentially useful species will be necessary to harness the full potential of seaweed resources and to do so sustainably.

4 Utilization of Seaweeds from Historical Period to Present Day

The earliest record of use of seaweeds dates back to 2700 BC in the compilation on “Chinese Herbs” by Emperor Shen Nung. Seaweeds have been a part of the Japanese diet since 300 BC. Seaweeds are mainly eaten in the oriental countries like Japan, China, Korea, and more recently in the USA, Europe, Philippines, Indonesia, Chile, Taiwan, Vietnam, Russia, Italy, and India. The Republic of Korea has the highest per capita consumption of seaweeds in the world. After human food consumption, the next most valuable commercial use of seaweeds is as a raw material for extraction of phycocolloids like agar, alginate, and carrageenan which are used in several industries. The current phycocolloids (seaweed gels) industry stands at over US $ 6.2 billion. The world production of commercial seaweeds has grown by 119 species since 1984, and presently, 221 species of seaweeds are utilized commercially including 145 species for food and 110 species for phycocolloid production. These have been used as food for human beings, feed for animals or manure for plants, and source of various chemicals. In the recent past, seaweeds have also been gaining new systems for biologist seaweed liquid fertilizer. Seaweed products are used in our daily lives in one way or another (e.g., seaweed polysaccharides are employed in the manufacture of toothpastes, soaps, shampoos, and cosmetic products such as creams and lotions and also as a source for animal nutrition). In addition, it is used in wastewater treatment, paper industry, and medical research (Cruz-Suarez et al. 2010). Recently, seaweed figure prominently debates about the energy and biofuel production. They have been discussed as a potential source against global warming where seaweeds and algae are very efficient carbon sinks which means that they absorb more carbon than their rate emission. Marine algae may also be used as energy collectors, and potentially useful substances may be extracted by fermentation and pyrolysis. The various benefits of different algal species available worldwide are presented in Table 1.

Table 1 Global utilization of different algal species

Species	Uses	Countries
Chlorophyta		
Acetabularia major	M	Indonesia, Philippines
Capsosiphon fulvescens	F	Korea
Caulerpa spp.	F	Malaysia, Thailand, India
Caulerpa lentillifera	F, M	Philippines
Caulerpa peltata	F, M	Philippines
Caulerpa racemosa	F	Bangladesh, Japan, Philippines South Pacific Islands, Vietnam, India
	M	Philippines, India
Caulerpa sertularioides	F, M	Philippines, India
Caulerpa taxifolia	F, M	Philippines, India
Codium spp.	F	Argentina
Codium bartletti	F	Philippines
Codium edule	F	Philippines
Codium fragile	F	Korea, Philippines
Codium muelleri	F	Hawaii
Codium taylori	F	Israel
Codium tenue	F	Indonesia
Codium tomentosum	F	Indonesia
Colpomenia sinuosa	F	Philippines
Dictyosphaeria cavernosa	Ag	Kenya
	M	Philippines
Enteromorpha spp.	Ag	Portugal, India, Brazil
	F	Bangladesh, France, Hawaii, Myanmar
Enteromorpha compressa	F	Korea, Indonesia
	M	Indonesia, Philippines
Enteromorpha clathrata	F	Korea
Enteromorpha grevillei	F	Korea
Enteromorpha intestinalis	F	Indonesia, Japan, Korea
	M	Indonesia
	Ag	India
Enteromorpha linza	F	Korea
Enteromorpha nitidum	F	Korea
Enteromorpha prolifera	F	Indonesia, Japan, Korea, Philippines
	M	Indonesia
Monostroma nitidum	F	Japan
Scytosiphon lomentaria	F	Korea, France
Ulva spp.	Ag	Italy, Portugal, India, New Zealand
	F, M	Argentina, Canada, Chile, Hawaii, Japan, Malaysia, India
	P	Italy
Ulva lactuca	F, M	Vietnam, Indonesia, India, Japan
Ulva pertusa	M	Philippines
Ulva reticulata	F	Vietnam, India
Rhodophyta		
Acanthophora spicifera	C	Vietnam
	F	Philippines, Vietnam
Ahnfeltia plicata	Ag	Chile
Asparagopsis taxiformis	F	Hawaii, Indonesia
	M	Philippines
Betaphycus gelatinum	F, C	Vietnam
Caloglossa adnata	F	Indonesia
Caloglossa leprieurii	M	Indonesia, Vietnam
Catenella spp.	F	Myanmar
Chondria crassicaulis	F	Korea
Chondrus crispus	C	France, Spain, USA
	F	Ireland, France
Chondrus ocellatus	F	Japan
Eucheuma alvarezii	C	Malaysia, Kiribati, India, Philippines
Eucheuma cartilagineum	F	Japan
Eucheuma denticulatum	C	Philippines, Madagascar
Eucheuma gelatinae	A	India, Malaysia, Vietnam
	C, F	China, Indonesia, Philippines
Eucheuma isiforme	F	Caribbean
Eucheuma muricatum	F, M	Indonesia
Eucheuma striatum	C	Madagascar
Gelidiella acerosa	F, A	Philippines, India, Malaysia, Vietnam
Gelidiella tenuissima	F	Bangladesh
Gelidium anansii	F, M	Korea, Indonesia
Gelidium abbottiorum	A	South Africa
Gelidium capense	A	South Africa
Gelidium chilense	A	Chile
Gelidium latifolium	A	Spain
	F	Indonesia
Gelidium lingulatum	A	Chile
Gelidium madagascariense	A	Madagascar
Gelidium pristoides	A	South Africa
Gelidium pteridifolium	A	South Africa
Gelidium pusillum	F	Bangladesh
Gelidium robustum	A	Mexico
Gelidium rex	A	Chile
Gelidium sesquipedale	A	Morocco, Portugal, Spain
Gelidium vagum	A	Canada
Gigartina canaliculata	C	Mexico

(continued)

Table 1 (continued)

Species	Uses	Countries
Gigartina chamissoi	C	Peru
	C	Chile
Gigartina intermedia	C	Vietnam
Gigartina skottsbergii	C	Argentina, Chile
Gloiopeltis spp.	F	Vietnam
Gloiopeltis furcata	F	Korea
	C	Japan
Gloiopeltis tenax	C	Japan
	F	Korea
Gloiopeltis complanata	C	Japan
Gracilaria spp.	Ag.	Portugal
	C	Malaysia
	F	Myanmar, Thailand, India
	P	Italy
	M	Vietnam
Gracilaria asiatica	A	China, Vietnam
	F	Vietnam
Gracilaria bursa-pastoris	F	Japan
Gracilaria caudata	A	Brazil
Gracilaria changii	F	Thailand
Gracilaria chilensis	A	Chile
	Ag	New Zealand
Gracilaria cornea	A	Brazil
	F	Caribbean
Gracilaria coronopifolia	F	Hawaii, Vietnam
Gracilaria crassissima	F	Caribbean
Gracilaria domingensis	F	Brazil, Caribbean, Chile
Gracilaria edulis	A, F	India
Gracilaria eucheumoides	F	Indonesia, Vietnam
	M	Indonesia
Gracilaria firma	A	Philippines, Vietnam
	C	Philippines
	F	Vietnam
Gracilaria fisheri	A, F	Thailand
Gracilaria foliifera	A	India
Gracilaria gracilis	A	Namibia, South Africa
Gracilaria heteroclada	A	Philippines, Vietnam
	F	Vietnam
Gracilaria howei	A	Peru
Gracilaria lemaneiformis	A	Mexico, Peru
	F	Japan
Gracilaria longa	A	Italy
Gracilaria pacifica	A	Canada
Gracilaria parvispora	F	Hawaii
Gracilaria salicornia	A	Thailand
	F	Thailand, Vietnam
Gracilaria tenuistipitata var.	A	China, Philippines, Thailand, Vietnam
	F	Thailand, Vietnam
Gracilaria verrucosa	A	Argentina, Egypt, Italy, India
	F	France, Indonesia, Japan, Korea
	M	Indonesia
Gracilariopsis lemaneiformis	A	Canada
Gracilariopsis tenuifrons	A	Brazil
Grateloupia filicina	F	Indonesia, Japan
	M	India
Gymnogongrus furcellatus	C	Chile
Halymenia spp.	F, Ag	Myanmar, China, West Indies
Halymenia discoidea	F	Bangladesh
Halymenia durvillaei	F	Philippines
Halymenia venusta	Ag, F	Kenya
Hypnea spp.	F	Myanmar
Hypnea musciformis	C	Brazil, India
Hypnea muscoides	C, F	Vietnam, India
Hypnea nidifica	F	Hawaii
Hypnea pannosa	F	Bangladesh, Philippines
Hypnea valentiae	C, F	Vietnam
Iridaea ciliate	C	Chile
Iridaea edulis	F	Iceland
Iridaea laminarioides	C	Chile
Iridaea membranacea	C	Chile
Kappaphycus alvarezii	C	Philippines, Tanzania, India
	F	Philippines
Kappaphycus cottonii	C, F, M	Vietnam
Laurencia obtusa	F, M	Indonesia
Laurencia papillosa	Ag, F	Kenya, Philippines, India
Laurencia pinnatifida	F	Portugal
Lithothamnion corallioides	Ag	France, Ireland, UK
Mastocarpus papillatus	C	Chile
Mastocarpus stellatus	C	Portugal, Spain
	F	Ireland
Mazzaella splendens	A, F	Canada
Meristotheca papulosa	F	Japan
Meristotheca procumbens	F	South Pacific islands
Nemalion vericulare	F	Korea

(continued)

Table 1 (continued)

Species	Uses	Countries
Palmaria hecatensis	F	Canada
Palmaria mollis	F	Canada
Palmaria palmata	F	Canada, France, Iceland, Ireland, UK, USA
Porphyra spp.	F	Israel, New Zealand, UK, Japan
Porphyra abbottae	F	Alaska, Canada
Porphyra acanthophora	F	Brazil
Porphyra atropurpurea	F, M	Indonesia
Porphyra columbina	F	Argentina, Chile, Peru
Porphyra crispata	F	Thailand, Vietnam
Porphyra fallax	F	Canada
Porphyra haitanensis	F	China
Porphyra kuniedae	F	Korea
Porphyra leucosticta	F	Portugal
Porphyra perforata	F	Canada
Porphyra psuedolanceolata	F	Canada
Porphyra seriata	F	Korea
Porphyra spiralis	F	Brazil
Porphyra suborbiculata	F	Korea, Vietnam
Porphyra tenera	F	Japan, Korea
Porphyra torta	F	Alaska, Canada
Porphyra umbilicalis	F	France, USA
Porphyra vietnamensis	F	Thailand
Porphyra yezoensis	F	China, Japan, Korea
Pterocladia capillacea	A	Portugal
Pterocladia lucida	A	New Zealand
	F	Korea
Scinaia moniliformis	F	Philippines
Solieria spp.	F	Myanmar
Phaeophyta	A	New Zealand
Alaria crassifolia	F	Japan
Alaria fitulosa	Ag, F	Alaska
Alaria marginata	F	Canada
Alaria esculenta	F	Iceland, Ireland, USA
Ascophyllum nodosum	Ag	France, Canada, China, Iceland, USA
	Al	Ireland, Norway, UK
Cladosiphon okamuranus	F	Japan
Cystoseira barbata	Al	Egypt
Desmarestia spp.	RoK	Alaska
Durvillaea antarctica	F	Chile, New Zealand
Durvillaea potatorum	Al	Australia
Ecklonia cava	F	Japan
Ecklonia maxima	Ag	South Africa
Ecklonia stolonifera	F	Korea
Egregia menziesii	F	Canada
Fucus spp.	Ag	France
Fucus gardneri	Ag	Canada
	F, RoK	Alaska
Fucus serratus	Al	Ireland
	F	France
Fucus vesiculosus	Al	Ireland
	Co	Ireland
	F	France, Portugal
Hizikia fusiformis	F	Japan, Korea
Hydroclathrus clathratus	Ag	Philippines
	F	Bangladesh, Philippines
Laminaria angustata	F	Japan, Australia
Laminaria bongardiana	F	Alaska
Laminaria diabolica	F	Japan
Laminaria digitata	Al	France, Ireland
	F	Ireland
Laminaria groenlandica	F	Canada
Laminaria hyperborean	Al	Ireland, Norway, Spain, UK
Laminaria japonica	Al	China
	F	China, Japan, Korea
Laminaria longicruris	F	USA
Laminaria longissima	F	Japan
Laminaria ochroleuca	Al	Spain
Laminaria ochotensis	F	Japan
Laminaria religiosa	F	Japan, Korea
Laminaria saccharina	F	Alaska, Canada, Ireland
	RoK	Alaska
Laminaria setchellii	F	Canada
Laminaria schinzii	Ag	South Africa
Lessonia nigrescens	Al	Chile, Peru
Lessonia trabeculata	Al	Chile
Macrocystis integrifolia	Al	Peru
	RoK	Alaska, Canada
Macrocystis pyrifera	Ag	Australia
	Al	Chile, Mexico, Peru, USA
	F	Argentina
	RoK	Alaska, USA
Nemacystis decipiens	F	Japan
Nereocystis luetkeana	Ag	Alaska, Canada
	F	USA
Pelvetia siliquosa	F	Korea
Postelsia spp.	F	USA
Sargassum aquifolium	F	Indonesia
Sargassum crassifolium	Al	Vietnam
	F	Thailand

(continued)

Table 1 (continued)

Species	Uses	Countries
Sargassum spp.	Ag	Brazil, Vietnam, India, China, South Africa, Australia
	Al	Vietnam
	F	Bangladesh, Hawaii, Malaysia, Myanmar, Philippines, Thailand, Vietnam
	M	Brazil, Vietnam
Sargassum filipendula	F	Egypt
Sargassum graminifolium	Al	Vietnam
Sargassum henslowianum	Al	Vietnam
Sargassum horneri	F	Korea
Sargassum ilicifolium	Al	India
Sargassum mcclurei	Al	Vietnam
Sargassum myriocystum	Al	India
Sargassum oligosystum	F	Thailand
Sargassum polycystum	F	Indonesia, Thailand
	AL, M	Vietnam
Sargassum siliquosum	Al	Vietnam
	F, M	Indonesia
Sargassum wightii	Al	India
Sargassum vachelliannum	Al	Vietnam
Turbinaria spp.	Ag	Vietnam
	M	Philippines
Turbinaria conoides	Al	India
Turbinaria decurrens	Al	India
Turbinaria ornata	Al	India
Undaria pinnatifida	F	Australia, China, France, Japan, Korea
Undaria peterseniana	F	Korea, Japan, China

F food, *A* agar, *C* carrageenan, *Al* alginate, *M* medicine, *RoK* roe on kelp, *Ag* agricultural, *P* paper (Zemke-White and Ohno 1999)

5 Seaweeds for Industrial Applications

5.1 Agar–Agar Production

Agar is the major constituent of the cell walls of certain red algae especially members of the Gelidiaceae and Gracilariaceae. Agar is widely used in paper manufacturing, culture media, packaging material, photography, leather industry, plywood manufacturing, cosmetics, and pharmaceutical industry.

5.1.1 Method of Agar Production from Seaweed

A brief account of the extraction of agar from *Gracilaria*/*Gelidium* is given below (McHugh 2003). Collected and dried seaweeds are washed with water to remove adhering mud and sand. It is soaked in acidified water (0.5 N HCl) for 24 h. It is then washed, taken, and introduced into boiling potable water at 100 °C. The ratio of seaweed to water is 1:40. The pH at the beginning of the extraction is adjusted to slightly acidic after introducing seaweeds. This will facilitate easy extraction of the gel. Extraction was carried out at 100 °C for 1 h at this temperature, and then the liquid is allowed to simmer for another hour. Finally, the extract is left in a warm chamber to cool gradually, permitting the sedimentation of suspended particles. The clear supernatant liquid was separated, kept at ambient temperature for some time, and then cooled. The gel is formed and is slightly warmed to melt. Or the gel is removed, melted in water, and poured in to enamel trays to form the gel again. Upper layer of the gel is sufficiently scored using a cutter to increase the surface area to enable to freeze immediately. It is frozen at temperature between 0 and −5 °C for 24 h and it is allowed to thaw at room temperature. The process is repeated till complete water is removed. It is then drained off; the gel is placed on plastic screen and then in galvanized wire netting and is dried either in shade or in hot air at 65 °C in most of the agar manufacturing plants, and it is bleached using mild chlorine water when it is thawed. It is washed free of chlorine using potable water. The dried agar is powdered and packed in polyethylene bags. Agar can also be packed in the form of agar shreds. The agar obtained is of good quality, and the yield is about 13–15 % of the dry seaweeds. The processing method is slightly different when the bacteriological grade is manufactured. Gel solution is filtered using a filter press and drying is done at a low temperature. It is pulverized using a pulverizer fine mesh. Gel strength is the most important criterion for the quality of agar. High gel strength is a must for the bacteriological grade of agar. Commercial agar obtained from the Indian *Gracilaria* by acid treatment process has got very low gel strength in the range of 200–250 g/cm^2. As the gel strength of agar produced is very low, it fetches very low price.

5.1.2 Carrageenan

Carrageenans obtained from various red algae like *Chondrus*, *Gigartina*, *Eucheuma*, and *Hypnea* are employed in food industries. It is valuable in the manufacture of "sausages," meatballs, ham, preparations of poultry and fish, chocolates, desert gel, ice creams, juice, textiles, toothpastes, hair shampoos, sanitary napkins, fungicides, etc. There are three main types of carrageenan such as lambda, kappa, and iota each having their own gel characteristics. Previously, the use of carrageenan was restricted because of the availability of natural resources of *Chondrus crispus* (common name: Irish moss) from Canada, Ireland, Portugal, Spain, and France and *Gigartina*/*Iridaea* from South America and Southern Europe (Trono 1997). *Chondrus* contains a mixture of two types (lambda and kappa) that could not be separated during commercial extraction. Limited quantities of wild *Chondrus* are still used; attempts to cultivate *Chondrus* in tanks have been biologically successful but uneconomic as a raw material for carrageenan (McHugh 2003). These sources now only contribute 20 % of the total processed material. Supplies for this phycocolloid are dominated by *Eucheuma* which are cultivated in Indonesia and the Philippines and recently very successfully in India and Tanzania (Trono 1997).

5.1.2.1 Production of Carrageenan

Washed sun-dried seaweed is boiled in a digester by passing a team and treating with 10 % KOH (caustic potash). The bleached seaweed is sun-dried or dried indoor on sieve plates below which hot air is passed. Then, the bleached and dried seaweed is grinded in a pulverizer to make into powder. Carrageenan is extracted from this crude (semi-processed) carrageenan similar to that of agar extraction by large-scale industry method, except treating the seaweed with 5–10 % sodium hydroxide solution before the hot extraction (Bixler 1996).

5.1.3 Alginate

Alginate is a polysaccharide extracted from brown seaweeds such as *Sargassum*, *Turbinaria*, *Dictyota*, and *Padina* generally growing in cold water areas worldwide and also occurring in the Indian waters. Alginate is used in the preparation of various pharmaceutical food and rubber products, textile productions, and others. This can readily form nontoxic gels (Chapman 1970). Alginates find their uses in varied industries, but the most important consumers are textile (50 %) and food (30 %) industries. As with other phycocolloids, various grades of alginate are available for specific applications and associated prices, e.g., sodium alginate in both pharmaceutical and food grade (McHugh 1987).

6 Medicinal Uses of Algae

Seaweeds were considered to be of medicinal value in the early as 3000 BC. Chinese and Japanese used them in the treatment of goiter and other diseases. Romans used the seaweeds for healing wounds, burns, and rashes. Though the importance of different seaweed products in pharmacology is known, the development of antibacterial, antifungal, and antiviral substances from seaweeds is still in the growing stages of research and development. Iodine is the most important element to enable the thyroid glands to secrete the thyroxin which contains 60 % iodine. It controls the general development of the animal. Seaweeds are the best source of iodine for human beings. Several important seaweed medicinal preparations are prepared in various countries, i.e., Kelpeck is prepared from kelps in Chicago, Burbank vegetable tablets are seaweed preparations from the USA, Kelpamalt is a seaweed medicinal preparation from New York (USA), Isokelp is prepared in California, and Parakelp and Manamar are other medicinal seaweeds (Chennubhotla et al. 2013).

Gelidium cartilagineum has been found to be against influenza B and mumps viruses. Among the seaweeds, red algae have been the major producer of bioactive secondary metabolites. Isolation of polysaccharides and other compounds with antiviral activity against enveloped viruses increased interest in algae as a source of antiviral compounds; subsequently, polysaccharides from extracts of red algae were to inhibit herpes simplex virus (HSV) and other viruses. Extracts from the California red algae *Schizymenia pacifica* contained a sulfated polysaccharide in the r-carrageenan family, which selectively inhibited HIV reverse transcriptase (Iwashima et al. 1999).

6.1 Medicinal Values of Red Algae

- *Alsidium helminthocorton*, *Digenea simplex*, and *Corallina officinalis* – vermifuge
- *Chondrus crispus* and *Gigartina stellata* – cough, chest, and stomach ailments
- *Porphyra* sp. and *Palmaria palmata* – antiscorbutic

6.2 Medicinal Values of Brown Algae

- *Fucus vesiculosus* – scrofula
- *Fucus evanescens* – stomach ailments
- *Laminaria* and other kelps – iodine source (cures goiter), stipes used to open wounds and in cervical dilation

6.3 Medicinal Values of Green Algae

- *Ulva* spp. – burn treatment
- *Acetabularia major* – bladder and kidney ailments

6.4 Antitumor Activity of Seaweeds

The anticancer property of different types of marine algae was extensively reviewed by Lee et al. (2013). An antitumor activity was tested on the different fraction of red alga *Porphyra telfairiae*. It was shown that the petroleum ether fraction showed an obvious inhibition effect on Sarconema 180 (S sub (180)), hepatic carcinoma (hep A), and enrich carcinoma (ECA) under the dose-dependent relation in the range of 125–500 mg kg^{-1}, whereas the inhibitory effects on tumor did not affect the increase of the body weight of mice. In addition, the petroleum fraction showed some cell toxicity HeLa cells and HL-60 cells. The chloroform and butanol fraction had a less effect compared with the petroleum ether fraction and water fractions and strong activity. Five compounds were isolated from petroleum ether fraction, and structures of four were elucidated as beta-sitosterol, stearic acid, Tetra triacontanol stearate, and beta-sitosterol palmitate on the basis of chemical and spectral evidence.

6.5 Sulfated Polysaccharides from Seaweeds

Fucan, a family of sulfated polysaccharides present in brown seaweeds, has several biological activities. Their use as drugs would offer the advantage of no potential risk of contamination with viruses or particles such as prions. A fucan prepared from *Spatoglossum schroederi* was tested as a possible inhibitor of cell–matrix interactions using wild-type Chinese hamster ovary cells (Cho-k1) and the mutant type deficient in xylosyltransferase (Cho-745). The efficient polymer on adhesion properties with specific extracellular matrix components was studied using several matrix proteins as substrates for cell attachment treatment with the polymer inhibiting the adhesion of fibronectin to both Cho-k1 (2 × 10.) and Cho-745 (2 × 10.) and 5 × 10. cells. No effect was detected with laminin using the cell types. On the other hand, adhesion to vitronectin was inhibited in Cho-k1 cells and adhesion to type -1 collagen was inhibited. The fucan did not affect either cell proliferation or cell cycle (Rocha et al. 2001).

6.6 Antihypercholesterolemic Activity

In the right and left sides of the peritoneal cavity, levels of total cholesterol and low-density lipoprotein decreased to 37 % and 24 % due to seaweed. For histochemical changes, hepatic tissues obtained at 40 h after injection of the triton and the *Porphyra yezoensis* extract were fixed in from calcium solution. The number of lipid drops and cholesterol particles decreased in the portal space of the hepatic cytoplasm. This indicated that the accumulation of lipid, including cholesterol, caused by triton was prevented by the antihypercholesterolemic effect of extract from the seaweed *P. yezoensis* (Hong et al. 1998).

6.7 Anticoagulant Substances from Seaweeds

An anticoagulant isolated from the marine green alga *Codium pugniformis* was composed mainly of glucose with minor amounts of arabinose and galactose. It was highly sulfated (326 µ mg polysaccharide) and contained protein (52 µ mg polysaccharide) and was thus a proteoglycan. The anticoagulant properties of the purified proteoglycan were compared with those of heparin by studying the activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) using normal human plasma. The proteoglycan showed similar activities to heparin, but was weaker than heparin. On the other hand, the proteoglycan did not affect PT even at the concentration at which APTT and TT were prolonged. The anticoagulation mechanism of this proteoglycan was due to the direct inhibition of thrombin and the potentiation of antithrombin III. Ethanol extracts from a group of 53 marine organisms were evaluated for their antimicrobial and antiparasitic activity. The activity against *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis* (Gram-positive), *Escherichia coli* (Gram-negative and Gram-positive), *Escherichia coli* (Gram-negative), and *Candida albicans* (yeast) was determined by the diffusion agar method. From this group, 15 ethanol extracts were tested against *Entamoeba histolytica*, and *Giardia lamblia*, *Lithothamnium crassiuscula*, *Geodia* sp., *Pacifigorgia* sp. showed significant activity against *Entamoeba histolytica*, while *Myxilla incrustans* and *Muricea appressa* were active against *Giardia lamblia. Lithothamnium crassiuscula* showed activity against both trophozoites (Matsubara et al. 2000).

6.8 Immunosuppressive Activity from Seaweeds

Water extracts of marine algae with immunosuppressive activity were investigated for in vivo activity using murine models of collagen-induced arthritis and skin transplantation. Eleven (three brown and eight red algae) of them had suppressive activity on the collagen-induced mouse arthritis model. Of these, *Eisenia bicyclis*, *Sargassum sagamianum*, *Amphiroa aberrans*, and *Gracilaria verrucosa*, in particular, showed high activity. On the other hand, treatment with extracts from *Codium fragile*, *C. intricatum*, *C. divaricatum*, and *Liagora* sp. prolonged the allograft survival time on the murine skin rejection model. One of these algal extracts, those from *Liagora* sp., markedly prolonged the allograft survival time. These results suggest that bioactive compounds with immunosuppressive activity may be contained in these algae (Mizukoshi et al. 1995).

6.9 Antiulcer Substance from Seaweeds

Porphyran inhibits Gram-negative bacterium *Helicobacter pylori* colonization. This substance can eliminate specifically *H. pylori* from the stomach and used in the prevention or treatment of gastritis, gastric ulcers, duodenal ulcers, and gastric cancer. Oral administration of porphyran prevents the adhesion of the urease on the *H. pylori* cells so as to prevent several diseases associated with it (Bhatia et al. 2003).

6.10 Agglutination, Coagulation, and the Stimulation of Cell Migration Properties

Macromolecule recognition processes are common in cells and their specificity is their most important characteristic. Many research programs exploit recognition events, and these have become the focus areas of research in biology, chemistry, medicine, and pharmacology. Biological reactions that involve recognition events include processes such as cell agglutination and coagulation, the stimulation of cell migration, and fertilization. Lectins, sometimes referred to as hemagglutinins or agglutinins, are glycoproteins with an ability to agglutinate red blood cells (Boyd and Reguera 1949). Various polysaccharides are present on cell surfaces and as a result many cells including microbes and yeasts. Tumor cells (Hori et al. 1986) and erythrocytes are selectively agglutinated by lectins (Chen et al. 1995).

Lectins are inhibited by sugars of the same type as those on the surface of the cells being agglutinated (Sharma and Sahni 1993). They are useful in exploring properties of biological structures and processes and have found applications in biology, cytology, biochemistry, medicine, and food science and technology. Lectins from *Codium* sp. have been developed into commercially available reagents and are routinely used in biochemical studies. Lectins with hemagglutinating properties occur in a verity of red, green, and brown algae (Rogers and Hori 1993; Shanmugam et al. 2002). They react with a wide array of erythrocytes including human blood group types. Agglutination reactions with human blood groups have led to their use in assays for blood typing. Lectins are also used to characterize cell-surface polysaccharides or to examine cell-binding patterns in lectinosorbent assays (Wu et al. 1998). Lectins from *Codium fragile* subspecies *tomentosoides* have been developed into a histochemical reagent by coupling them to colloidal gold, forming a lectin-gold conjugate. This conjugate is useful for studies of the surface topography of cells of animal tissues (Griffin et al. 1995).

6.11 Antilipemic, Hypocholesterolemic, Hypoglycemic, Hypotensive, and Related Activities

High plasma cholesterol levels and high blood pressure are the causes of cardiovascular disease. Some macroalgal polysaccharides and fibers such as alginate, carrageenan, funoran, fucoidan, laminaran, porphyran, and ulvan have been noted to produce hypocholesterolemic and hypolipidemic responses due to reduced cholesterol absorption in the gut (Panlasigui et al. 2003). This is often coupled with an increase in the fecal cholesterol content and a hypoglycemic response (Dumelod et al. 1999). Others have reported lowering of systolic blood pressure (antihypertensive responses) and lower levels of total cholesterol, free cholesterol, triglyceride, and phospholipid in the liver (Nishide and Uchida 2003). Evidence suggests that ulvan as a dietary fiber plays a protective role in the rate such that it modulates the stimulatory effect of mucin secretion by goblet cells into the colon (Barcelo et al. 2000). A crude methanolic extract from *Pelvetia babingtonii* showed potent α-glucosidase inhibitory activity which could make it effective in suppressing postprandial hyperglycemia (Ohta et al. 2002).

Hypolipidemic activities have been identified in ethanolic extracts of *Solieria robusta*, *Lyngarisa stellata*, *Colpomenia sinuosa*, *Spatoglossum asperum*, and *Caulerpa racemosa*, as shown by decreases in the total serum cholesterol, triglyceride, and low-density lipoprotein cholesterol levels in rats (Ara et al. 2002). PGE2 from *Gracilaria lichenoides* has antihypertensive properties when administered intravenously to hypertensive rats. Some of these substances, most notably the fibers, are likely to be exploited by nutraceutical companies that market them as health products.

6.12 Antioxidants from Seaweeds

Marine algae are constantly exposed to various environmental conditions including freezing, carbon limitation, water stress, and heat stress.

These conditions are the main causes for the formation of ROS and contribute to the photoinhibition of photosynthesis. The brown alga *Fucus evanescens* produced ROS due to freezing, high light, and desiccation stress (Collén and Davison 1999, 2001) which was detected with fluorescent dyes (Collén and Davison 1997). *Mastocarpus stellatus*, a red alga, showed higher antioxidant activity other than red seaweed *Chondrus crispus,* because of its daily exposure to high air temperature in the lower intertidal zone. The activities of enzymatic and nonenzymatic antioxidants increase with tidal height and also with temperature. In the high tidal levels, the marine algae are continuously exposed to both visible and ultraviolet radiation, resulting in higher ROS production.

7 Utilization of Seaweed as Fertilizer

The use of seaweeds as manure in farming practice is very ancient and was prevalent among the Romans and also practiced in Britain, France, Spain, Japan, and China (Thirumaran et al. 2009). Seaweed manure is a slow but long-activating fertilizer, and its application is well suited to light sandy soils, which are generally deficient in potash. Physical condition of these light soils also improves (crumb structure) on account of the gelatinous nature of seaweeds. This is attributed to the high content of polysaccharides and its consequent capacity for holding of water. Seaweed extracts are used extensively in agriculture as plant growth supplements and seaweed meal takes months to become fully effective in the soil as a plant nutrient. Seaweed concentrates are known to cause many beneficial effects on plants as they contain growth-promoting hormones (IAA and IBA, cytokinins) trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), vitamins, and amino acids (Challen and Hemingway 1965). Hence, large quantities of seaweeds can be used as manure in all parts of the country, either directly in the form of compost. Seaweed fertilizer application improves the fertility of soils in cultivated fields particularly the brown seaweeds because of their alginate content, which helps in conditioning the soil facilitating aeration, moisture retention, and absorption of nutrient elements. Seaweed liquid fertilizer (SLF) application (spraying) sometimes reduces the incidence of insect attack, and sugar beet and potato leaves treated with seaweed extract had significantly fewer levels infested with aphids (20 %) than the untreated leaves (83 %). The application of SLF to improve the growth of terrestrial plants is fast becoming an accepted practice. In general, the reported beneficial effects of seaweeds are improvement of overall plant vigor, yield, quality, and quantity of different plant parameters which is able to withstand any adverse environmental conditions (Balakrishnan et al 2007). Unlike, chemical fertilizers, extracts derived from seaweeds are biodegradable, nontoxic, nonpolluting, and nonhazardous to humans, animals, and birds (Anandhan and Sorna kumari 2011).

7.1 Methods of Application

The seaweed fertilizers can be prepared by the methods of manure (or) compost, crude manure, and liquid preparation. Generally, seaweed extracts are applied in small dosages. It is clear that the active ingredients in seaweed extracts are effective in low concentrations with Hoagland solution protocol followed by Epstein (1972).

7.1.1 Manure (or) Compost

Seaweeds have been used as a food and manure for plantation crops by coastal people in many countries (Kaliaperumal et al. 1987). Recent research suggests that application of seaweed extract as seed treatment and/or foliar spray helps significant growth of plants. The extract contains micronutrients, auxins and cytokinins, and other growth-promoting substances (Spinelli et al. 2010). Seaweed and its derived products are used as fertilizer in the coastal areas throughout the globe. In India, it is used for coconut plantations especially in Tamil Nadu and Kerala (Kalimuthu et al. 1987). The high amount of water-soluble potash and other mineral and trace elements present in seaweeds is readily absorbed by plants and they control nutrient deficiency in plants. Carbohydrates and other organic matter present in seaweeds alter the nature of the soil and improve its moisture retaining capacity. Hence, large quantities of seaweeds including sea grasses such as *Cymodocea*, *Diplanthera*, *Enhalus*, and *Halophila* are used as manure in all parts of the country either directly or in the form of compost.

7.1.2 Seaweed Liquid Fertilizer (SLF)

Seaweed extracts exhibit growth-stimulating property on crop plants. Hence, its formulation can be used as a bio-stimulant in agriculture. The bio-stimulant present in seaweed extract increases the vegetative growth (10 %), the leaf chlorophyll content (11 %), the stomata density (6.5 %), the photosynthetic rate, and the fruit production (27 %) of the plant (Spinelli et al. 2010). In spite of the proven capability of SLF on growth and yield promotion of various crops, the extraction procedure from seaweeds, its concentration, and mode of application have not been standardized. The liquid seaweed extracts from seaweeds are usually prepared by hydrolyzing the material under pressure; however, the preparation may vary from species to species depending upon the amount of dried material available. The method of extraction significantly differs from person to person and also the mode of application to crops. Seaweed extracts are used in several ways, such as drench in soil during transplantation, during field preparation (Lingakumar et al. 2002), seed treatment (Immanuel and Subramanian 1999), or as foliar application.

Foliar applications of liquid fertilizer supply the plant with nutrients more rapidly than methods involving uptake by root due to seed/root treatment. Growers, therefore, can apply SLF as foliar treatment to quickly correct nutrient deficiencies. Foliar treatment has some drawbacks, mainly due to the structure of the leaf and the temporary nature of the nutrient supply. Leaves, particularly those with thick cuticle, have low absorption rates. Therefore, multiple applications of liquid fertilizers are necessary to supply a sufficient quantity of the nutrients to the plants. Further, once applied, foliar nutrients may be washed off by rain or irrigation water before the plant absorbs them. To counter this loss, surfactants can be used to increase the efficiency of penetration of the leaf surface and the duration of the sprays on the leaf be increased depending upon the situation. At certain cases, application of high nutrient concentrations in foliar spray causes severe leaf damage due to phytotoxicity. To avoid this situation, repeated applications of dilute formulations, therefore, is necessary to supply the plant's nutrient requirements without damaging the foliage. Since there are different types of seaweed extracts available in the market, it is important for the farmer/grower to know the type of species used in preparation of SLF and how to properly use it for specific crops. The timing, dosage, and frequency of application are very important when dealing with seaweed extract. Application rate and frequency may vary based on location, time of season, soil type, and crop. Proper application is important because higher concentration of seaweed extract may damage the plant resulting to loss in yields (Spinelli et al. 2010).

7.2 Present Status of Seaweed Fertilizer Usage

Though seaweed and its derived product are increasingly used in the production of agricultural crops, the mechanism of action of seaweed extract on enhancement of productivity is still unknown. The recent challenge in sustainable food production is due to the increasing occurrence of biotic and abiotic stress due to climate change, which may lead to the reduction of agricultural productivity globally. Under this situation, SLF may work as a good inducer for sustainability in agricultural production coupled with maintenance of soil health. In India, seaweeds are not used extensively except for production of phycocolloids. However, being a rich source of vitamins, minerals, and growth promoters, they can be of immense help to the coastal farmers for their use as a source of organic fertilizer. Hence, there is a need for popularizing the use of seaweed as health food and liquid organic fertilizer through mass scale field trials and organization of public awareness programs (Mohanty et al. 2013).

8 Utilization of Seaweeds as Food

Seaweeds are considered as a food supplement in the twenty-first century because they contain proteins, lipids, polysaccharides, minerals, vitamins, and enzymes. In general, seaweeds are rich in vitamins A, E, C, and niacin with similar contents in green algae (Chlorophyta), brown algae (Phaeophyta), and red algae (Rhodophyta). The concentration of vitamins B12, B1, pantothenic acid, and folic and folinic acids is generally higher in greens and reds than in browns (Madlener 1977). The brown algae possess organic iodine in greater amounts, whereas in green algae, this micronutrient is found generally in low quantity. For example, one tablespoon of cooked hijiki (*Hizikia fusiforme*), a brown algae, is approximately equivalent in calcium to one glass of whole milk. On the other hand, tea made from *Fucus vesiculosus* (bladder wrack) is called “slimming tea” because the high iodine content in the plants will act as a stimulator of the thyroids that regulates the metabolism, and there is no better way to provide the body with a full complement of trace elements than consuming these kinds of sea vegetables. Marine algae are similar to oats in protein and carbohydrate values. The red and green algae appear higher in crude protein far tested about 2–4 %. For example, the blue-green algae *Nostoc* species have around 20 % protein content, which is similar for the green algae *Enteromorpha linza* (20 %) and the brown algae *Analipus japonicus* (22 %). The protein values of red algae *Porphyra* are higher than rice or soybeans and very close to horsemeat meal (Madlener 1977).

All algae contain high content of carbohydrates (sugars and starches) in polysaccharide biochemical structure which is a natural nontoxic colloidal substance that has been used as mucilaginous material referred to as gel. However, this structure cannot be broken by the digestive enzymes in several organisms, and therefore their use for human consumption is nutritionally limited (Madlener 1977). Fat content in sea vegetables ranges from 1 % in *Laminarias* to 8 % in *Pelvetia canaliculata*.

8.1 Food Source from Green Seaweeds

The green seaweeds *Monostroma*, *Enteromorpha*, *Ulva*, *Caulerpa*, and *Codium* are commonly known as source of food. In Japan, dried fronds of edible *Monostroma* are used in preparation of “nori-jam” and soup. Edible *Monostroma* and *Enteromorpha* are called “Aonori” in Japanese (Ohno 1997); in some Pacific regions, *Enteromorpha* is being known as “ele ele” (Hawaii), “Iulua,” “lumi boso” (Fiji), and “Nalumlum malekesa” (Vanuatu). This alga is being eaten by humans as edible raw, dried, or cooked (Novaczek 2001). *Codium geppiorum* is a favorite dish with fish cooked in milk by many Pacific islanders. *Caulerpa* is known as “sea grapes,” “green caviar,” or “green sea feather.” It is commonly sold in markets and is important to the economy of many Pacific regions. *Caulerpa lentillifera* is being consumed as a salad in the Philippines and some parts in Indonesia (Trono and Toma 1997), and *C. sertularioides*, *C. peltata*, and *C. bikinensis* are being consumed with coconut milk (Payri et al. 2000).

8.2 Food Source from Brown Seaweeds

Laminaria “kombu” and *Undaria* “wakame” are edible and important resources in Japan. They are consumed raw, boiled, or dried material with sweetened green beans, jelly, crushed ice, and coconut milk in Southern Vietnam (Tsutsui et al. 2005). *Cladosiphon okamuranus* is consumed as salad in Japan (Toma 1997). *Sargassum* is known as horsetail, and it is eaten as soup or dressed with soybean sauce, after being processed in Korea (Madleners 1977) and in Hawaii (Novaczek 2001). In the Pacific region, *Rosenvingea* or slippery cushion and *Turbinaria* or spiny leaf are eaten as soup or omelet; *Colpomenia* or papery sea bubble as chop soup, stew, or salad; and *Hydroclathrus* or sea colander, *Dictyota* or brown, and Padina or sea fan ribbon weeds as a food dressing, soup, or stew (Novaczek 2001).

8.3 Food Source from Red Seaweeds

Acanthophora or spiny sea plant, *Asparagopsis* or supreme limu, *Callophyllis* or large wire weed, *Hypnea* or maidenhair, *Halymenia* or red sea lettuce, *Laurencia* or flower limu, and *Scinaia* or tender golden weed are eaten fresh or raw; chopped and cooked, especially with coconut milk, or sprinkled as a spice in salads; used to make pudding and jellies; and dried and rehydrated in the Pacific regions (Novaczek 2001).

Gracilaria or sea moss is being used as homemade agar, garnish for sashimi, used for commercial agar, or fresh as a salad (Madlener 1977; Novaczek 2001). *Gelidiella* or little wire weed is eaten after being simmered as a jelly in Japan and Vietnam (Madlener 1977; Novaczek 2001; Tanaka and Nakamura 2004). *Rhodymenia palmata* or dulse is the most common of edible seaweeds in Europe and North America. *Alaria fistula*, *Chordaria flagelliformis*, and *Porphyra umbilicalis* are also used as food, while *Porphyra* or purple lever is being consumed fresh or dried in Japan, China, Korea, Vietnam, North America, and Europe (Madlener 1977; Tanaka and Nakamura 2004; Tsutsui et al. 2005). *Eucheuma* and *Kappaphycus* or thorn grass, elkhorn (*Eucheuma*), and brown licorice algae tambalang (*Kappaphycus*) are being eaten with coconut milk and sugar in Indonesia and Vietnam (Tsutsui et al. 2005).

8.4 Seaweed Recipes

It is known that about 100,000 tones of seaweeds are eaten annually in Japan in the name nori, kombu, and hakama. Seaweeds are rich in proteins, vitamins, amino acids, growth hormones, minerals, and other trace elements. Hypothyroidism (goiter) can be controlled by the intake of iodine-rich seaweeds like *Asparagopsis taxiformis*, *Sarconema* spp., etc. Indian seaweed can be best consumed as follows: *Caulerpa sertularioides*, *Codium*, *Gracilaria confervoides*, *Hydroclathrus clathratus*, *Laurencia papillosa*, and *Hypnea valentiae* as seaweed salad; *Ulva lactuca* as seaweed masala; *Gracilaria edulis* as seaweed pickle, seaweed wafer, and seaweed jelly; and *Ulva lactuca* as seaweed jam (Chennubhotla et al. 1981).

8.5 Production of Vegetable Oils from Microalgae

Most current research on oil extraction is focused on microalgae to produce biodiesel from algal oil. The biodiesel from algal oil in itself is not significantly different from biodiesel produced from vegetable oils. Dilution, microemulsification, pyrolysis, and transesterification are the four techniques applied to solve the problems encountered with high fuel viscosity. Of the four techniques, transesterification of oil into its corresponding fatty ester (biodiesel) is the most promising solution to the high viscosity problem. This is accomplished by mixing methanol with sodium hydroxide to make sodium methoxide. This liquid is then mixed into vegetable oil. The entire mixture then settles and glycerin is left on the bottom while methyl esters, or biodiesel, is left on top. Biodiesel can be washed with soap and glycerin using a centrifuge and then filtered. Kinematic viscosities of the fatty acid methyl esters vary from 3.23 to 5.61 mm/s (Knothe 2005). Methanol is preferred for transesterification because it is less expensive than ethanol (Graboski and McCormick 1998). For production of biodiesel, macroalga (*Cladophora fracta*) and microalga (*Chlorella protothecoides*) samples were used (Demirbas 2008). The higher heating value of *Chlorella protothecoides* (25.1 MJ/kg) is also higher than that of *Cladophora fracta* (21.1 MJ/kg). Most vegetable oils are unsaturated. The properties of the various individual fatty esters that comprise biodiesel determine the overall fuel properties of the biodiesel fuel. The average polyunsaturated fatty acids of *Chlorella protothecoides* (62.8 %) are also higher than those of *Cladophora fracta* (50.9 %). Algae generally produce a lot of polyunsaturates, which may present a stability problem since higher levels of polyunsaturated fatty acids tend to decrease the stability of biodiesel. However, polyunsaturates also have much lower melting points than monounsaturates or saturates; thus, algal biodiesel should have much better cold weather properties than many other bio-oils (Demirbas 2008). A large amount of microalgal oil was efficiently extracted from the heterotrophic cells using n-hexane and then transmuted into biodiesel by acidic transesterification (Xu et al. 2006).

9 Seaweeds Used as Animal Feed

Seaweeds had been used for many years directly for human consumption and animal feed. It is also an ingredient for the global food and cosmetics industries and is used as fertilizer and as an animal feed additive. Also, seaweeds are valuable sources of food, micronutrients, and raw materials for the pharmaceutical industry. Seaweed has plenty of essential nutrients, especially trace elements and several other bioactive substances that explains why seaweeds are considered as a food supplement in the twenty-first century and as source of proteins, lipids, polysaccharides, mineral, vitamins, and enzyme (Rimber 2007). Interestingly, the best known component of the seaweed-derived industry is that of the phycocolloids, the gelling, thickening, emulsifying, binding, stabilizing, clarifying, and protecting agents known as carrageenans, alginates, and agars (Chopin 2007). Total annual use by the global seaweed industry is about 8 million tones of wet seaweed (McHugh 2003).

9.1 Seaweed Used as Fish Feed

In fish farming, wet feed usually consists of meat waste and fish waste mixed with dry additives containing extra nutrients all formed together in a doughy mass. When thrown into the fishponds or cages, it must hold together and not disintegrate or dissolve in the water. A binder is needed; sometimes a technical grade of alginate is used. It has also been used to bind formulated feeds for shrimp and abalone. However, the use of finely ground seaweed meal made from brown seaweeds is cheaper.

There is also a market for fresh seaweed as a feed for abalone. In Australia, the brown seaweed *Macrocystis pyrifera* and the red seaweed *Gracilaria edulis* have been used. In South Africa, *Porphyra* is in demand for abalone feed, and recommendations have been made for the management of the wild population of the seaweed. Pacific dulse (*Palmaria mollis*) has been found to be a valuable food for the red abalone, *Haliotis rufescens*, and development of land-based cultivation has been undertaken with a view of producing commercial quantities of the seaweed. The green seaweed, *Ulva lactuca*, has been fed to *Haliotis tuberculata* and *H. discus* (McHugh 2003). Feeding trials showed that abalone growth is greatly improved by high protein content, and this is attained by culturing the seaweed with high levels of ammonia present.

9.2 Seaweed Used as Feed for Farm Animals

Seaweeds are cheap sources of minerals and trace elements besides vitamins, growth hormones, and phycocolloids. Hence, meals prepared from seaweeds can be given as supplements to the daily rations of the cattle, poultry shrimps, and fishes. Seaweed meal can be mixed with fish meal and used as poultry feed. Dave et al. (1977) assessed the possibility of seaweeds being used as supplementary animal feed and they reviewed the feeding trails of animals with seaweeds conducted in Japan, Germany, the UK, Norway, and other countries.

9.3 Seaweed Utilization in Integrated Aquaculture

Cultivation of *Gracilaria* started in Taiwan Province of China in the 1960s as a source of raw material for its agar industry. At first cultivation was on ropes in ditches containing fishpond effluents, but in 1967, it was moved into the fishponds themselves. This had the twofold benefit of the seaweed using the fish waste material as fertilizer and the fish eating the epiphytes, such as *Enteromorpha* species, that would otherwise become serious pests for the seaweeds. Control with tilapia (*Oreochromis mossambicus*) and milkfish (*Chanos chanos*) was satisfactory as long as the fish were removed before they started to eat the *Gracilaria*; larger fish were periodically removed and replaced by small fish. This concept of polyculture, or integrated aquaculture to use the more recent terminology, has since been utilized in many situations where the effluent from the aquaculture of one species, potentially threatening environmental damage, can be utilized by another species to its advantage, with a reduction in pollution. Various strategies have been tried. Seaweed cultivation around the outside of fish cages has led to significantly better growth of seaweed but was only partly successful in removing the large amount of nutrients coming from the fish cages. Unattached *Gracilaria* has been grown in the effluent from shrimp ponds. Semi-enclosed or land-based systems have been suggested, but the higher capital investment has been a deterrent (McHugh 2003).

Integrated aquaculture is developing as solutions are sought to problems of environmental sustainability, including the management of coastal areas and the disposal of effluents from large-scale aquaculture activities. Animal aquaculture techniques affect adversely the environment in one way or another (Ackefors and Enell 1990). They generate increased sedimentation, biochemical oxygen demand, nutrient loadings, etc., inherent to highly intensive stocking and feeding. Wastewater contains a large amount of nitrogen excreted by the animals as particulate or in the dissolved state (del Rio et al. 1996). The worldwide increase during the last few years on mono-species aquaculture has generated severe environmental problems and is a matter of great concern. Haglund and Pedersén (1993) investigated the potential of the red seaweed *Gracilaria tenuistipitata* when cocultivated with *Oncorhynchus mykiss* (rainbow trout) for the removal of nitrogen and phosphorus from the pond. Integrated management of seaweed in shrimp aquaculture ponds is a common practice in China and Taiwan since it attributes the following: (1) the seaweeds could be a suitable shelter for the animals especially during the day; (2) the oxygen evolved during photosynthesis by seaweed helps the aerobic bacteria to accelerate the degradation of complex organic substances to simple elements; (3) ammonia, urea, and other nutrients present in the excreta of the animals are being utilized by the seaweed for its productivity thereby reducing the nutrient loading; (4) the polysaccharides and other products obtained from the seaweed while grown in aquaculture farms exhibit good quality since the ambient water is enriched with nutrients; (5) the shrimp farmers benefited not only from the animals but also from the seaweeds; and (6) the level of oxygen during the day is increased due to photosynthesis. However, both animals and plants compete for oxygen during night. Therefore, maintenance of stocking density of seaweed in the pond is a prerequisite for integrated aquaculture practice (Kavitha and Rengasamy 2002).

10 Utilization of Seaweed Biomass for Fuel

Continued use of petroleum sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of CO_2 in the environment. Renewable, carbon-neutral transport fuels are necessary for environmental and economic sustainability (Chisti 2007). Biodiesel can be carbon neutral and produced intensively on relatively small areas of marginal land. The quality of the fuel product is comparable to petroleum diesel and can be incorporated with minimal change into the existing fuel infrastructure. Innovative techniques, including the use of industrial and domestic waste as fertilizer, could be applied to further increase biodiesel productivity (Campbell 2008). Similar to higher plants like corn, soybeans, sugar cane, wood, and other plants, algae also used photosynthesis to convert solar energy into chemical energy. They store this energy in the form of oils, carbohydrates, and proteins. The plant oil can be converted into biodiesel; hence, biodiesel is a form of solar energy.

Algae are among the fastest growing plants in the world, and about 50 % of their weight is oil. This lipid oil can be used to make biodiesel for cars, trucks, and airplanes. Microalgae have much faster growth rates than terrestrial crops. The per-unit area yield of oil from algae is estimated to be between 20,000 and 80,000 L/acre/year; this is 7–31 times greater than the next best crop, palm oil (Chisti 2007). It is possible that US demand for liquid fuel could be achieved by cultivating algae in one tenth the area currently devoted to soybean cultivation (Scott and Bryner 2006). The lipid and fatty acid contents of microalgae vary in accordance with culture conditions. Most current research on oil extraction is focused on microalgae to produce biodiesel from algal oil. Algal oil can be processed into biodiesel as easily as oil derived from land-based crops.

The production of microalgal biodiesel requires large quantities of algal biomass. The algae that are used in biodiesel production are usually aquatic unicellular green algae. This type of algae is a photosynthetic eukaryote characterized by high growth rates and high population densities. Under good conditions, green algae can double their biomass in less than 24 h (Schneider 2006; Chisti 2007). Additionally, green algae can have huge lipid contents, frequently over 50 % (Schneider 2006; Chisti 2007). This high-yield, high-density biomass is ideal for intensive agriculture and may be an excellent source for biodiesel production. A one ha algae farm on wasteland can produce over 10–100 times as much oil compared to any other known source of oil crops. While a crop cycle may take from 3 months to 3 years for production, algae can start producing oil within 3–5 days, and thereafter oil can be harvested on a daily basis. Algae can be grown using sea water and non-potable water on wastelands where nothing else grows. Algae farming for biofuels is expected to provide a conclusive solution to the food vs. fuel debate. The production of biodiesel has recently received much attention worldwide. In order to resolve the worldwide energy crisis, seeking for lipid-rich biological materials to produce biodiesel effectively has attracted much renewed interest. Algae have emerged as one of the most promising sources for biodiesel production. It can be inferred that algae grown in CO_2-enriched air can be converted into oily substances. Such an approach can contribute to solving the major problems of air pollution, resulting from CO_2 emissions and future crises due to a shortage of energy sources (Sharif Hossain et al. 2008).

The process for producing microalgal oils consists of a microalgal biomass production step that requires light, CO_2, water, and inorganic nutrients. The latter are mainly nitrates, phosphates, iron, and some trace elements. Approximately half of the dry weight of microalgal biomass is carbon, which is typically derived from CO_2. Therefore, producing 100 tons of algal biomass fixed roughly 183 tons of CO_2. This CO_2 must be fed continually during daylight hours. It is often available at little or no cost (Chisti 2008). The optimal temperature for growing many microalgae is between 293 and 303 K. A temperature outside this range could kill or otherwise damage the cells. There are three well-known methods to extract oil from algae: (1) expeller/press, (2) solvent extraction with hexane, and (3) supercritical fluid extraction. A simple process is to use a press to extract a large percentage (70–75 %) of the oils from algae. Algal oil can be extracted using chemicals. The most popular chemical for solvent extraction is hexane, which is relatively inexpensive. Supercritical fluid extraction is far more efficient than traditional solvent separation methods. Supercritical fluids are selective, thus providing the high purity and product concentrations (Paul and Wise 1971). This method alone can allow one to extract almost 100 % of the oils. In supercritical fluid CO_2 extraction, CO_2 is liquefied under pressure and heated to the point where it has the properties of both a liquid and a gas. This liquefied fluid then acts as the solvent in extracting the oil. The lipid and fatty acid contents of microalgae vary in accordance with culture conditions. Algal oil contains saturated and monounsaturated fatty acids. The fatty acids exist in algal oil in the following proportions: 36 % oleic (18:1), 15 % palmitic (16:0), 11 % stearic (18:0), 8.4 % isolinoleic (17:0), and 7.4 % linoleic (18:2). The high proportion of saturated and monounsaturated fatty acids in this alga is considered optimal from a fuel quality standpoint, in that fuel polymerization during combustion would be substantially less than what would occur with polyunsaturated fatty acid-derived fuel (Sheehan et al. 1998). Oil levels of 20–50 % are quite common (Chisti 2007; Carlsson et al. 2007; Demirbas 2009). After oil extraction from algae, the remaining biomass fraction can be used as a high protein feed for livestock (Schneider 2006; Haag 2007). This gives further value to the process and reduces waste. Moreover, according to the biodiesel standard published by the American Society for Testing Materials (ASTM), biodiesel from microalgal oil is similar in properties to standard biodiesel and is also more stable according to their flash point values.

10.1 Economics of Biodiesel Production

There are small numbers of economic feasibility studies on microalgae oil (Richardson et al. 2009). Currently, microalgae biofuel has not been deemed economically feasible compared to the conventional agricultural biomass (Carlsson et al. 2007). Critical and controversial issues are the potential biomass yield that can be obtained by cultivating macro- or microalgae and the costs of producing biomass and derived products. The basis of the estimates is usually a discussion of three parameters: photosynthetic efficiency, assumptions on scale up, and long-term cultivation issues. For microalgae, the productivity of raceway ponds and photobioreactors is limited by a range of interacting issues. Typical productivity for microalgae in open ponds is 30–50 t/ha/year (Benemann and Oswald 1996; Sheehan et al. 1998). Several possible target areas to improve productivity in large-scale installations have been proposed (Benemann and Oswald 1996; Grobbelaar 2000; Suh and Lee 2003; Torzillo et al. 2003; Carvalho et al. 2006). Harvesting costs contribute 20–30 % to the total cost of algal cultivation, with the majority of the cost attributable to cultivation expenses. Genetic engineering, development of low-cost harvesting processes, improvements in photobioreactor, and integration of coproduction of higher-value products/processes are other alternatives in reducing algal oil production costs (Chisti 2007). The harvested algae then undergo anaerobic digestion, producing methane that could be used to produce electricity. In commercial photobioreactors, higher productivities may be possible. Typical productivity for a microalga (*Chlorella vulgaris*) in photobioreactors is 13–150 (Pulz 2001). Photobioreactors require ten times more capital investment than open pond systems. The estimated algal production cost for open-pond system is $ 10/kg and photobioreactors are from $ 30 to $ 70/kg. The cost of algal production is two to three orders of higher magnitude than conventional agricultural biomass (Carlsson et al. 2007). Assuming that biomass contains 30 % oil by weight and carbon dioxide is available at no cost (flue gas), Chisti (2007) estimated the production cost for photobioreactors and raceway ponds at $ 1.40 and $ 1.81/L of oil, respectively. However, for microalgal biodiesel to be competitive with petrodiesel, algal oil should be less than $ 0.48/L (Chisti 2007).

It is useful to compare the potential of microalgal biodiesel with bioethanol from sugar cane, because on an equal energy basis, sugarcane bioethanol can be produced at a price comparable to that of gasoline (Bourne Jr. 2007). Bioethanol is well established for use as a transport fuel (Gray et al. 2006), and sugarcane is the most productive source of bioethanol (Bourne Jr. 2007). For example, in Brazil, the best bioethanol yield from sugarcane is 7.5 m^3/ha (Bourne Jr. 2007). However, bioethanol has only ~64 % of the energy content of biodiesel. Therefore, if all the energy associated with 0.53 billion m^3 of biodiesel that the USA needs annually (Chisti 2007) were to be provided by bioethanol, nearly 828 million m^3 of bioethanol would be needed. This would require planting sugarcane over an area of 111 million ha or 61 % of total available US crop land. Recovery of oil from microalgal biomass and conversion of oil into biodiesel are not affected by whether the biomass is produced in raceways or photobioreactors. Hence, the cost of producing the biomass is the only relevant factor for a comparative assessment of photobioreactors and raceways for producing microalgal biodiesel.

If the annual biomass production capacity is increased to 10,000 t, the cost of production per kilogram reduces to roughly $ 0.47 and $ 0.60 for photobioreactors and raceways, respectively, because of economies of scale. Assuming that the biomass contains 30 % oil by weight, the cost of biomass for providing a liter of oil would be something like $ 1.40 and $ 1.81 for photobioreactors and raceways, respectively (Chisti 2007). Biodiesel from palm oil costs roughly $ 0.66/L, or 35 % more than petrodiesel. This suggests that the process of converting palm oil into biodiesel adds about $ 0.14/L to the price of oil. For palm oil-sourced biodiesel to be competitive with petrodiesel, the price of palm oil should not exceed $ 0.48/L, assuming no tax on biodiesel. Using the same analogy, a reasonable target price for microalgal oil is $ 0.48/L for algal diesel to be cost competitive with petrodiesel.

10.2 Improving Economics of Microalgal Biodiesel

Algae are among the fastest growing plants in the world, and about 50 % of their weight is oil. That lipid oil can be used to make biodiesel for cars, trucks, and airplanes. Algae will someday be competitive as a source of biofuel. Only renewable biodiesel can potentially completely displace liquid fuels derived from petroleum. The economics of producing microalgal biodiesel need to improve substantially to make it competitive with petrodiesel, but the level of improvement necessary appears to be attainable (Demirbas 2008). Biodiesel has great potential; however, the high cost and limited supply of renewable oils prevent it from becoming a serious competitor with petroleum fuels. As petroleum fuel costs rise and supplies dwindle, biodiesel will become more attractive to both investors and consumers. For biodiesel to become the alternative fuel of choice, it requires an enormous quantity of cheap biomass. Using new and innovative techniques for cultivation, algae may allow biodiesel production to achieve the price and scale of production needed to compete with, or even replace, petroleum (Campbell 2008).

It has been estimated that 0.53 billion m^3 of biodiesel would be needed to replace current US transportation consumption of all petroleum fuels (Chisti 2007). Neither waste oil nor seed oil can come close to meeting the requirement for that much fuel; therefore, if biodiesel is to become a true replacement for petroleum, a more productive source of oil such as algal oil is needed (Scott and Bryner 2006; Chisti 2007). The cost of producing microalgal biodiesel can be reduced substantially by using a biorefinery-based production strategy, improving capabilities of microalgae through genetic engineering and advances in photobioreactor engineering. Like a petroleum refinery, a biorefinery uses every component of the biomass raw material to produce usable products (Chisti 2007).

11 Utilization of Seaweeds in Wastewater Treatment

11.1 Seaweeds in Sewage Treatment

Wastewater stabilization ponds are designed for anaerobic and aerobic bacteria and algae to decompose waterborne organic wastes efficiently. Ludwig et al. (1951) studied the role of algae in the treatment of sewage by photosynthetic oxygenation in waste stabilization ponds. Indian coastal waters are constantly polluted with industrial and domestic wastes. Domestic sewage is rich in nutrients such as NH_4-N, NO_2-N, NO_3,-N, PO_4-P, K, etc. The discharge of sewage into the coastal waters is estimated to be 35 Km^3/year (Qasim and Sen Gupta 1988). Addition of such enormous quantities of pollutants into the coastal water affects its water quality and cause eutrophication. Therefore, treatment of wastewaters before their discharge into the sea is essential. Red seaweeds like *Chondrus crispus*, *Gracilaria foliifera*, and *Neoagardhiella baileyi* when grown in the aquaculture system considerably removed the nutrients (Ryther et al. 1979). Dhargalkar (1986) observed that *Ulva fasciata* and *G. verrucosa* showed better growth in 5 % sewage seawater mixture. The biomass of *Ulva sp.* and *Enteromorpha sp.* increased when they were grown near the vicinity of discharge of domestic effluent. The use of *Ulva* sp. as biofilters has been suggested as an efficient method to recover large amount of dissolved inorganic nitrogen (Vandermeulen and Gordin 1990). Some seaweeds are used to remove heavy metals to clean up wastewater. Milled, dried species of the brown seaweeds *Ecklonia*, *Macrocystis*, and *Laminaria* were able to adsorb Cu, Zn, and Cd ions from the solution. In another laboratory-scale trial, *Ecklonia maxima*, *Lessonia flavicans*, and *Durvillaea potatorum* adsorbed Cu, Ni, Pb, Zn, and Cd ions, though to varying extents depending on the seaweed type and metal ion concentration. After the extraction of alginate from brown seaweeds, there is an insoluble waste product, mostly cellulose, and the adsorbing properties of this have been tested and found to equal some of the brown seaweeds. Using such a waste material is obviously more attractive than using the dried seaweed itself. Another waste product, from the production of Kelpak, and liquid fertilizer previously mentioned, has also been tested and found that it adsorbs Cu, Cd, and Zn just as effectively as the seaweed from which it is derived. So, there is the potential to use either seaweed or residues remaining from seaweed extraction. It is a matter of whether this is the most economical way to do so, depending on their availability and cost at the source of the wastewater (McHugh 2003). There are two main areas where seaweeds have the potential for use in wastewater treatment. The first is the treatment of sewage and some agricultural wastes to reduce the total nitrogen- and phosphorus-containing compounds before the release of these treated waters into rivers or oceans. The second is for the removal of toxic metals from industrial wastewater.

11.2 Treatment of Wastewater to Reduce Nitrogen- and Phosphorus-Containing Compounds

The rapid expansion of aquaculture has contributed to the excessive increase of nutrients, especially nitrogen and phosphorous, in aquatic ecosystems (Beveridge 1996). These nutrients generally originate from pond fertilization, feed, and metabolic residues of the cultivated animals. In this sense, the major challenge has been to develop nonpolluting strategies that minimize the negative effects of this activity. The most practical and economical approach to reduce the concentration of nutrients in aquaculture areas is to treat the effluents before it reaches the sea. A potentially feasible alternative is the biological treatment of effluents, using macroalgae for nutrient removal (Chopin et al. 2001; Neori et al. 2004). Heavy metals like Fe, Zn, Ca, and Mg have been reported to be of bio-importance to man and their daily medicinal and dietary allowances (Duruibe et al. 2007). Even for those having bio-importance, dietary intake has to be maintained at regulatory limits, as excesses will result in poisoning or toxicity, which is evident by certain reported medicinal symptoms that are clinically diagnosable (Fosmire 1990; Nolan 2003). Methods for removing metal ions from aqueous solution mainly consist of physical, chemical, and biological technologies. Seaweeds are presented as very good sorbents, because the cell wall of green and brown algae contains alginate with its carboxyl and hydroxyl groups (Davis et al. 2003; Vieira and Volesky 2000). Worse sorptive properties are suggested for red algae owing to its carrageen, exposing hydroxyl and sulfonate groups (Tsezos and Volesky 1981).

12 Conclusions

Ecological and commercial seaweeds are important as a potential source as food supplement in the twenty-first century because they serve as sources of proteins, lipids, polysaccharides, minerals, vitamins, and enzymes. The use of seaweeds in the development of pharmaceuticals, nutraceuticals, and cosmetics and as source of pigments, bioactive compounds, and antiviral agents is extensively discussed. They are being used for food, medicine, industry, and others such as integrated aquaculture with fishes, biofuel, and in removing the heavy metal in cleaning wastewater. Hence, seaweeds are the promising and versatile source for maintaining green environment in a sustainable manner.

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Part II

Renewable Energy

A Comprehensive Overview of Renewable Energy Status in India

Atul Sharma, Jaya Srivastava, and Anil Kumar

1 Introduction

Energy is considered a prime agent in the generation of wealth and a significant factor in economic development. Energy is also essential for improving the quality of life. Development of conventional forms of energy for meeting the growing energy needs of society at a reasonable cost is the responsibility of the Government. India is a burgeoning economy with an ever expanding need for energy. During the recent decades the rate at which energy demand has grown in India is one of the fastest in the world. India currently ranks as the world's seventh largest energy producer, accounting for about 2.49 % of the world's total annual energy production. It is also the world's fifth largest energy consumer, accounting for about 3.45 % of the world's total annual energy consumption in 2004 (Planning Commission 2013). This rapid growth in demand can be attributed to the rise in population and increasing economic development. Energy is a catalyst for economic growth. Hence, there is a need to generate and efficiently transmit more and more energy to all parts of the country.

Increasing demand and use of energy brings with it challenges of pollution and environmental degradation. At the same time rising crude oil prices are posing a problem of affordability for the common man. Conventional sources of energy are no longer sufficient to feed this power-hungry nation. The need of the hour is to make the energy basket of the country more broad based and to decrease the nation's dependence on coal and fossil fuels. Renewable energy is therefore being explored as an emerging viable solution to the energy problems of the nation.

1.1 What Is Renewable Energy?

Renewable energy is called "renewable" because the sources harnessed to create the energy renew and replenish themselves constantly and within a reasonably short period of time (i.e., months or years, not centuries). These sources of energy include water, wind, sun, biomass, and heat from the Earth's interior. The term renewable energy excludes energy created by nuclear fuels, such as uranium, and fossil fuels (oil, gas, and coal) because fossil fuels take millions of years to form and, once removed, require as many years to form again.

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The term renewable energy is not always synonymous with what is often called "green" energy. Typically, green energy refers to energy from renewable sources that leave smaller environmental footprints than conventional large-scale generation, including some renewable energy sources. For instance, although they utilize renewable energy and do not contribute to air pollution, some large-capacity hydroelectric projects require huge dams and reservoirs, which flood thousands of hectares of wilderness and disrupt the migration patterns of fish and wildlife. In contrast, some low-capacity, run-of-the-river hydroelectric projects use the flow of the water as it runs downstream to generate electricity and may result in little disruption to the environment or to local ecosystems.

1.2 Why Renewable Energy and Why Now?

All of the countries in the world are taking a new interest in renewable energy for several reasons. First, electricity from renewable energy produces fewer greenhouse gas emissions, which are associated with changing climate, than electricity produced from burning fossil fuels. Similarly, renewable energy generally adds fewer other pollutants to the air, including the following:

- Sulfur dioxide and nitrogen oxides that form acid rain
- Particulate matter, which along with ground-level ozone forms smog on hot, sunny summer days
- Mercury, which can be transformed in the environment to become highly toxic to people and animals

The supply of renewable energy is not only virtually unlimited (at the right price); it also offers the possibility of relatively stable prices. In the late 1990s and early years of the twenty-first century, all countries in the world watched as the prices of oil and gas soared, plummeted, and then soared again, in part because of the weather and in part because of world politics. Increasing the use of locally generated renewable energy can help protect us from dramatic price swings.

Table 1 Current status of the renewable energy in India (http://www.mnre.gov.in)

Renewable energy program/systems	Cumulative achievement up to 31.12.2012
I. Power from renewables:	
A. Grid-interactive power (capacities in MW)	
Wind power	18,420.40
Small hydropower	3,496.14
Biomass power	1,248.60
Bagasse cogeneration	2,239.63
Waste to power—urban/industrial	96.08
Solar power (SPV)	1,176.25
Total	**26,677.10**
B. Off-grid/captive power (capacities in MW_{EQ})	
Waste to energy—urban/industrial	113.60
Biomass (non-bagasse) cogeneration	426.04
Biomass gasifiers—rural	16.696
Biomass gasifiers—industrial	138.90
Aerogenerators/hybrid systems	1.74
SPV systems (>1 kW)	106.33
Water mills/micro hydel	2,121 Nos.
Total	**803.306**

MWeq. Megawatt equivalent, *MW* megawatt

Another major incident that has brought into focus the need for safer and cleaner energy options is the tsunami that struck Japan in 2011. The havoc created by the tsunami and the huge nuclear disaster at the Fukushima power plant have raised serious doubts about the use of radioactive materials for energy generation in our country. India is prone to earthquakes, and the large coastlines make the country vulnerable to the threats of tsunami. After the disastrous situation at the Daiichi power plant in Japan, there have been calls for reassessment of nuclear power projects in our country. Thus, it appears that renewable sources of energy can be a simpler, safer, and more cost-effective method of power generation. Table 1 shows the current status of renewable energy in India.

1.3 Current Renewable Energy Scenario

Changes in renewable energy markets, investments, industries, and policies have been so

Table 2 Selected top five countries for installations of renewable energy power projects

Top five countries	1	2	3	4	5
Annual amounts for 2011					
New capacity investment	China	USA	Germany	Italy	**India**
Wind power added	China	USA	**India**	Germany	UK/ Canada
Solar PV capacity	Italy	Germany	China	USA	France
Solar water heater/heat capacity	China	Turkey	Germany	**India**	Italy
Ethanol production	USA	Brazil	China	Canada	France
Biodiesel production	USA	Germany	Argentina	Brazil	France
Existing capacity as of 2011					
Renewables power capacity (including hydro)	China	USA	Brazil	Canada	Germany
Renewables power capacity (not including hydro)	China	USA	Germany	Spain	Italy
Wind power	China	USA	Germany	Spain	**India**
Biomass power	USA	Brazil	Germany	China	Sweden
Geothermal power	USA	Philippines	Indonesia	Mexico	Italy
Solar PV capacity	Germany	Italy	Japan	Spain	USA
Solar hot water/heat capacity	China	Turkey	Germany	Japan	Brazil

Renewables Global Status Report (2013)

rapid in recent years that perceptions of the status of renewable energy can lag years behind reality. According to the Renewable Energy Five-Year Plan (2008–2012) proposed by the Government of India, the renewable energy market in India will reach an estimated US $19 billion. Investments of US $15 billion will be required in order to add approximately 15,000 megawatts (MW) of renewable energy to the presently installed capacity (Renewable Energy World 2013). Renewable energy in 2010 supplied an estimated 16.7 % of global final energy consumption. Table 2 shows the list of the top five countries which have made significant strides in installations of renewable energy power projects.

2 Renewable Energy in India

India depends heavily on coal and oil for meeting its energy demand which contributes to smog, acid rain, and greenhouse gases emission. The last 25 years have been a period of exuberant hunt of activities related to research, development, production, and distribution of energy in India.

Fortunately, India has been blessed with plenty of alternate energy sources such as solar, wind, hydro, biomass, etc. Renewable energy represents an area of tremendous opportunity for India. Vigorous efforts during the past two decades are now bearing fruit as people in all walks of life are becoming more aware of the benefits of renewable energy, especially decentralized energy which is required in villages and in urban or semi-urban centers.

Energy “self-sufficiency” was identified as the major driver for new and renewable energy in the country in the wake of the two oil shocks of the 1970s. The sudden increase in the price of oil, uncertainties associated with its supply, and the adverse impact on the balance of payments position led to the establishment of the Commission for Additional Sources of Energy (CASE) in the Department of Science and Technology in March 1981. The commission was charged with the responsibility of formulating policies and their implementation and programs for the development of new and renewable energy apart from coordinating and intensifying R&D in the sector. In 1982, a new department, i.e., Department of Non-conventional Energy Sources (DNES), which incorporated CASE, was created in the Ministry of Energy. In 1992, DNES became the Ministry of Non-conventional Energy Sources (MNES), the world's first ministry committed to renewable energy. In October 2006, the ministry was rechristened as the Ministry of New and Renewable Energy (MNRE) (www.mnre.gov.in). Indian government policy framework in renewable energy generation is extremely investor friendly, and an attractive tariff and regulatory regime provide a strong foundation for the growth of the sector.

2.1 Solar Energy

Solar energy is the most abundant permanent energy resource on earth, and it is available for use in its direct (solar radiation) and indirect (wind, biomass, hydro, ocean, etc.) forms. Solar energy, experienced by us as heat and light, can be used through two routes: thermal route or the photovoltaic (PV) route. The thermal route uses the heat for water heating, cooking, drying, water purification, and power generation. The photovoltaic route converts the light in solar energy into electricity; it can be used for applications such as lighting, pumping, communications, and electrification of villages.

India lies in the sunny belt of the world. The scope for generating power and thermal applications using solar energy is huge. Most parts of India get 300 days of sunshine a year, which makes the country a very promising place for solar energy utilization (TERI 2001). The daily average solar energy incident over India varies from 4 to 7 kWh/m^2 with the sunshine hours ranging between 2,300 and 3,200 per year, depending upon location (MNES 2001). The technical potential of solar energy in India is huge. The country receives enough solar energy to generate more than 500,000 TWh per year of electricity, assuming 10 % conversion efficiency for PV modules. It is three orders of magnitude greater than the likely electricity demand for India by the year 2015 (Muneer et al. 2005). Figure 1 shows a map of India with solar radiation levels in different parts of the country. It can be observed that although the highest annual global radiation is received in Rajasthan, northern Gujarat, and parts of Ladakh region, the parts of Andhra Pradesh, Maharashtra, and Madhya Pradesh also receive fairly large amounts of radiation as compared to many parts of the world especially Japan, Europe, and the USA where development and deployment of solar technologies is maximum (Garud and Purohit 2009).

Solar power is the generation of electricity from sunlight. This can be direct as with photovoltaics (PV) or indirect as with concentrating solar power (CSP), where the sun's energy is focused to boil water which is then used to provide power (Sharma 2011). Since solar radiation is intermittent, solar power generation is combined either with storage or other energy sources to provide continuous power.

Concentrating solar power (CSP) systems use lenses or mirrors and tracking systems to focus a large area of sunlight into a small beam. The concentrated heat is then used as a heat source for a conventional power plant. A wide range of concentrating technologies exists; the most developed are the parabolic trough, the concentrating linear Fresnel reflector, the Stirling dish, and the solar power tower. Various techniques are used to track the sun and focus light. In all of these systems, a working fluid is heated by the concentrated sunlight and is then used for power generation or energy storage (Martin and Goswami 2005). Concentrated solar power systems can be used for a range of applications depending upon the energy conversion utilized, electricity or heat. However, at present, most systems focus on electricity generation. The parabolic trough collector is the best solution for applications in the low temperature ranges such as detoxification, liquid waste recycling, and heating water. All three systems are suitable for the mid-temperature range applications, and the central tower is the most suitable for high temperature range system because temperatures of more than 1,000 °C can be easily sustained.

Since solar power is at the introductory stage of its life cycle, Indian government initiatives are expected to drive it until 2012. The Government of India realizing the need for alternate sources of energy other than coal and oil has introduced

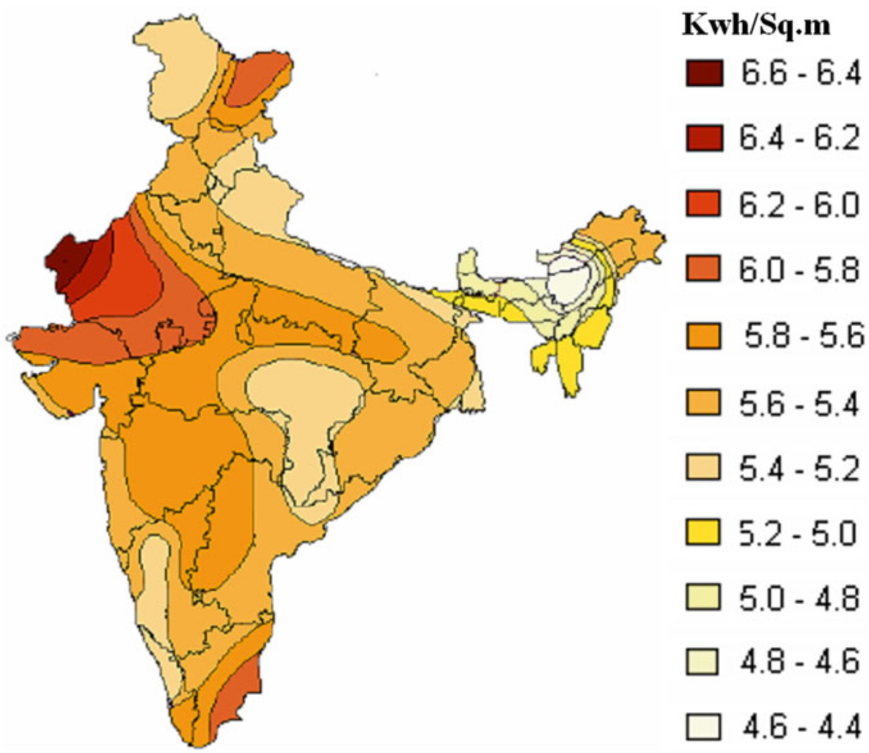

Fig. 1 Solar radiation on India (Garud and Purohit 2009)

many schemes and incentives to support the growth of the solar energy sector. The Government of India has recently launched the ambitious "Jawaharlal Nehru National Solar Mission (JNNSM)" which aims to promote the development and use of solar energy for power generation and other uses in the country (MNRE 2013). India's National Action Plan on Climate Change (2008) articulates a central role for solar power. The Government of India's National Action Plan on Climate Change released in mid-2008 by the Prime Minister's Council on Climate Change identifies eight critical missions, one of which is the National Solar Mission. The mission has twin objectives—to contribute to India's long-term energy security as well as its ecological security. The objective of the National Solar Mission is to establish India as a global leader in solar energy by creating the policy conditions for its diffusion across the country as quickly as possible. The Mission will adopt a three-phase approach, spanning the remaining period of the 11th Plan and first year of the 12th Plan (up to 2012–2013) as Phase 1, the remaining 4 years of the 12th Plan (2013–2017) as Phase 2, and the 13th Plan (2017–2022) as Phase 3. At the end of each plan, and midterm during the 12th and 13th Plans, there will be an evaluation of progress and review of capacity and targets for subsequent phases based on emerging cost and technology trends, both domestic and global. The aim would be to protect the government from subsidy exposure in case the expected cost reduction does not materialize or is more rapid than expected. The immediate aim of the mission is to focus on setting up an enabling environment for solar technology penetration in the country both at a centralized and decentralized level. The mission includes a major initiative for promoting rooftop solar photovoltaic (PV) applications. The solar tariff announced by the regulators will be applicable for such installations. The power distribution companies will be involved in the purchase of this power. The mission would have a "much focused R&D

program" which seeks to address India-specific challenges in promoting solar energy.

The amount of solar energy produced in India is less than 1 % of the total energy demand. Government-funded solar energy in India only accounted for approximately 6.4 MW/year of power as of 2005. However, India is ranked number one in terms of solar energy production per watt installed, with an insolation of 1,700–1,900 kilowatt hours per kilowatt peak. The grid-interactive solar power as of December 31, 2012 was merely 1,176 MW, and India expects to install an additional 10,000 MW by 2017 (MNRE 2013).

2.2 Wind Energy

Wind is a form of renewable energy. It is abundant, low cost, and widely distributed; it scales up easily and can be developed quickly. Winds are generated by complex mechanisms involving the rotation of the earth, heat energy from the sun, the cooling effects of the oceans and polar ice caps, temperature gradients between land and sea, and the physical effects of mountains and other obstacles. This wind flow, or motion energy, when "harvested" by modern wind turbines, can be used to generate electricity. Wind energy is a clean, eco-friendly, renewable resource and is nonpolluting to generate electricity. The use of electricity has grown since it can be used in a variety of applications; it can be easily transmitted as well as. Hence, the use of wind for generating electricity is rising. Wind turbines convert the kinetic energy in the wind into mechanical power. This mechanical power can be used for specific tasks (such as grinding grain or pumping water), or a generator can convert this mechanical power into electricity to power homes, businesses, schools, and the like.

India is blessed with plenty of alternate energy sources such as solar, wind, hydro, and biomass. India occupies the fifth position in the world after China, USA, Germany, and Spain in generation of wind power. Although we have seen an impressive increase in installed capacity addition, from barely about 1,350 MW at the time of independence (1947) to about 160,000 MW today, over 90,000 MW of new generation capacity is required in the next 7 years. The development of wind power in India began in the 1990s and has significantly increased in the last few years.

The total potential for wind power in India was first estimated by the Centre for Wind Energy Technology (C-WET) at around 45 GW and was recently increased to 48.5 GW. This was adopted by the government as the official estimate. The C-WET study was based on a comprehensive wind mapping exercise initiated by MNRE, which established a country-wide network of 1,050 wind monitoring and wind mapping stations in 25 Indian states. The gross wind power potential is estimated at around 48,561 MW in the country, a capacity of 18,420 MW up to December 31, 2012 has so far been added through wind, which places India in the fifth position globally (MNRE 2013).

Table 3 shows the cumulative installed capacity of the top 10 countries (The World Wind Energy Association 2012). The worldwide wind capacity reached 254,000 MW by the end of June 2012, out of which 16,546 MW were added in the first 6 months of 2012. This increase represents 10 % less than in the first half of 2011 when 18,405 MW were added. Still the five leading countries, China, USA, Germany, Spain, and India, represent together a total share of 74 % of the global wind capacity.

2.3 Biomass

Biomass has been a key player in energy generation even in the past. Biomass, defined as all land- and water-based vegetation as well as organic wastes, fulfilled almost all of human kind's energy needs prior to the industrial revolution. In present day scenario, once again its utilization for generation of energy has gained momentum because of the limited availability of conventional energy resources as well as environmental concern due to greenhouse gas emissions.

In recent years, the interest in using biomass as an energy source has increased, and it represents approximately 14 % of the world's final

Table 3 Top ten cumulative installed wind energy capacities during 2009–2010

Position	Country	By June 2012 (MW)	First half 2012 (MW)	End 2011 (MW)
1	China	67’774	5’410	62’364
2	USA	49’802	2’883	46’919
3	Germany	30’016	941	29’075
4	Spain	22’087	414	21’673
5	India	**17’351**	1’471	15’880
6	Italy[a]	7’280	490	6’787
7	France[b]	7’182	650	6’640
8	UK	6’840	822	6’018
9	Canada	5’511	246	5’265
10	Portugal	4’398	19	4’379
	Rest of the world	35’500	3’200	32’227
	Total	254’000	16’546	237’227

The World Wind Energy Association (2012)

[a] Till end of May 2012

[b] Till end of April 2012

energy consumption (India 2009). Estimates have indicated that 15–50 % of the world’s primary energy use could come from biomass by the year 2050. The major reasons for this are as follows: Firstly, technological developments relating to conversion, crop production, etc., promise the application of biomass at lower cost and with higher conversion efficiency than was possible previously. Secondly, the energy obtained from biomass is a form of renewable energy, and, in principle, when produced by sustainable means, biomass emits roughly the same amount of carbon during conversion as is taken up during plant growth. The use of biomass therefore does not contribute to a buildup of CO_2 in the atmosphere.

Biomass resources suitable for energy production covers a wide range of materials, from firewood collected in farmlands and natural woods to agricultural and forestry crops grown specifically for energy production purposes. Energy production from food wastes or food processing wastes, especially from waste edible oils, seems to be attractive based on bio-resource sustainability, environmental protection, and economic consideration. India is very rich in biomass and has a potential of 16,881 MW (agro-residues and plantations), 5,000 MW (bagasse cogeneration), and 2,700 MW (energy recovery from waste). Biomass power generation in India is an industry that attracts investments of over INR 600 crores every year, generating more than 5,000 million units of electricity and yearly employment of more than ten million man-days in rural areas (Singh and Gu 2010).

2.4 Hydropower

Hydropower is another source of renewable energy that converts the potential energy or kinetic energy of water into mechanical energy in the form of water mills, textile machines, etc., or as electrical energy (i.e., hydroelectricity generation). It refers to the energy produced from water (rainfall flowing into rivers, etc.). Hydropower is the largest renewable energy resource being used for the generation of electricity. India is endowed with hydro resources which are both viable and economically exploitable. In fact, hydropower is the second highest contributor of the energy consumed within India in the power sector. Large hydropower projects are being utilized for power production and account for most of the energy consumed which comes from renewable sources.

Countries like Norway, Canada, and Brazil have all been utilizing more than 30 % of their hydro-potential, while on the other hand India and China have lagged far behind. India ranks fifth in terms of exploitable hydro-potential in the world. According to CEA (Central Electricity Authority), India is endowed with economically exploitable hydropower potential to the tune of 1,48,701 MW (KPMG 2007).

As hydropower has been tapped and used for grid purposes, small hydropower will be focused on in this discussion, as small hydropower has small-scale applications which would benefit the energy deprived. In India, small hydro plants have a capacity of 25 MW or less and are further subdivided into micro (100 kW or less), mini (between 100 kW and 2 MW), and small (between 2 and 25 MW). Large hydropower is not covered in this chapter. Small hydropower plants (SHP) can be set up on rivers, canals, or at dams and are flexible in terms of installation and operation. These projects are considered environmentally benign, particularly when compared to large hydro plants with storage reservoirs, which can cause habitat destruction and community displacement (Palsh 2002). These projects are in fact even compatible with use of water used for irrigation and even drinking water. The cost of SHP projects depends on where they are set up, i.e., the location and the site's topography.

MNRE has estimated the potential for small hydro in India at 15,384.15 MW for 5,718 prospective plant sites published in MNRE's 2010–2011 Annual Report, which notes that efforts to identify additional prospective sites are ongoing in both the public as well as the private sector (MNRE Annual Report 2011). The estimated potential for small hydro in India also suggests that it can make a significant role in India's power supply, especially in remote areas where alternative supply solutions face lots of challenges. For these reasons, the further development of small hydro is one of the focal areas of MNRE that wants to concentrate on reducing the capital costs and enhancing the reliability, plant load factors, and average plant lifetimes. The Indian government's aim is that at least 5,000 MW of capacity is added from small hydro projects in the next 10 years and is supporting small hydro deployment through capital subsidies and preferential tariffs. As of December 2012, a total of 3,496.14 MW of grid-connected small hydropower has been installed, contributing about 13.11 % to India's total grid-interactive renewable power (MNRE 2012). Most of the potential is in Himalayan states as river-based projects and in other states on irrigation canals. Out of this potential, about 50 % lies in Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, and Uttarakhand. In the plain regions Chhattisgarh, Karnataka, Kerala, and Maharashtra have a sizeable potential. Continuous efforts are being made to identify new potential SHP sites. The ministry is providing financial support to the states for identification of new potential sites and preparation of a perspective plan for the state for development of small hydro projects.

2.5 Geothermal

Geothermal is energy generated from heat stored in the earth or the collection of absorbed heat derived from underground. Immense amounts of thermal energy are generated and stored in the Earth's core, mantle, and crust. Heat from the Earth or geothermal—Geo (Earth) + thermal (heat)—energy can be and is accessed by drilling water or steam wells in a process similar to drilling for oil. Geothermal energy is an enormous, underused heat and power resource that is clean (emits little or no greenhouse gases), reliable (average system availability of 95 %), and homegrown (making us less dependent on foreign oil) (EAI 2013).

Geothermal resources range from shallow ground to hot water and rock several miles below the Earth's surface and even farther down to the extremely hot molten rock called magma. Mile-or-more-deep wells can be drilled into underground reservoirs to tap steam and very hot water that can be brought to the surface for use in a variety of applications. The provinces, although found along the west coast in Gujarat and Rajasthan and along a west, south, west-east-northeast line running from the west coast to the western border of Bangladesh (known as SONATA), are most prolific in a 1,500 km stretch of the Himalayas. The resource is little used at

Table 4 Status of geothermal sites in India

Geothermal field	Estimated (min.) reservoir Temp (Approx)	Status
Puga geothermal field (Ladakh)	240 °C at 2,000 m	From geochemical and deep geophysical studies (MT)
Tattapani Surguja (Chhattisgarh)	120–150 °C at 500 m and 200 °C at 2,000 m	Magnetotelluric survey done by the National Geophysical Research Institute (NGRI). Hyderabad
Tapovan Chamoli (Uttarakhand)	100 °C at 430 m	Magnetotelluric survey done by NGRI
Cambay Graben (Gujarat)	160 °C at 1,900 m (from oil exploration borehole)	Steam discharge was estimated 3,000 cu meter/day with high-temperature gradient
Badrinath Chamoli (Uttarakhand)	150 °C estimated	Magnetotelluric study was done by NGRI. Deep drilling required to ascertain geothermal field
Surajkund Hazaribagh (Jharkhand)	110 °C	Magnetotelluric study was done by NGRI. Heat rate 128.6 mW/m^2
Manikaran Kullu (Himachal Pradesh)	100 °C	Magnetotelluric study was done by NGRI. Heat flow rate 130 mW/m^2
Kasol Kullu (Himachal Pradesh)	110 °C	Magnetotelluric study was done by NGRI

the moment but the government has an ambitious plan to more than double the current total installed generating capacity by 2012.

In India, exploration and study of geothermal fields started in 1970. The GSI (Geological Survey of India) has identified 350 geothermal energy locations in the country. The most promising of these is in Puga valley of Ladakh. The estimated potential for geothermal energy in India is about 10,000 MW (India Energy Portal 2013). But yet geothermal power has not been exploited at all, owing to a variety of reasons, the chief being the availability of plentiful coal at cheap costs. However, with increasing environmental problems with coal-based projects, India will need to start depending on clean and eco-friendly energy sources in the future; one of which could be geothermal. There are no operational geothermal plants in India. Table 4 shows the status of geothermal sites in India. Chandrasekhar and Chandrasekharam (2010) estimates an installed capacity of 200 MW (thermal) with an annual energy use of 1,600 TJ/year and a capacity factor of 25 %. It is expected that the geothermal sources can be used for low-grade heating and direct utilization in various commercial industries. In Ladakh, the northeastern part of Kashmir, demonstrations of heat pumps are planned, and a 50 MW power plant is planned in the Puga valley. Figure 2 shows the potential for geothermal energy in India.

2.6 Biofuels

Liquid biofuels, namely, ethanol and biodiesel, are used to substitute petroleum-derived transportation fuels. India's biofuel strategy is focused on using nonfood sources for the production of biofuels such as sugar molasses and nonedible oilseeds as well as second-generation biofuels in the near future. Advanced conversion technologies for ethanol are under development, which will allow it to be made from forest and agricultural residues. Using one-third of the surplus, biomass could yield about 19 billion liters of ethanol, which could displace the country's entire gasoline consumption once techno-economically viable. Advanced feedstock for biodiesel production includes microalgae, which is currently under research and has a very promising potential in India—it can provide double the yield of the highest producing crop (oil palm) per land unit (Indian Renewable Energy Status 2010).

India's commercial production of biodiesel is very small, and what is produced is mostly sold

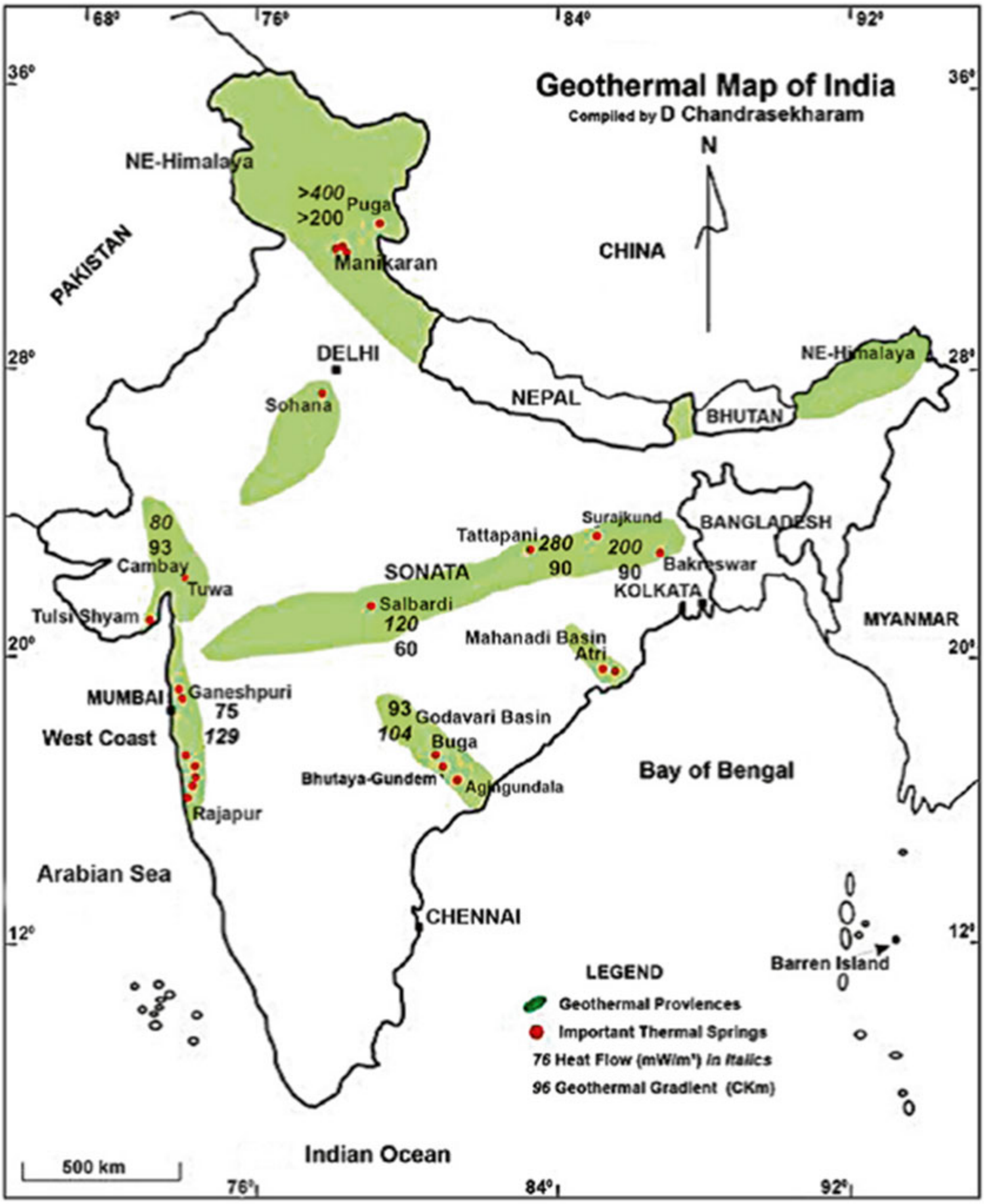

Fig. 2 Geothermal potentials in India (WHEC 2008)

for experimental projects and to the unorganized rural sector. Although India's biodiesel processing capacity is currently estimated at 200,000 tons per year, the majority of biodiesel units are not operational during most of the year. The existing biodiesel producers in India are using nonedible oilseeds, nonedible oil waste, animal fat, and used cooking oil as feedstock (USDA-FAS 2009).

Biodiesel production efforts in India are focused on using nonedible oils since the demand for edible oils exceeds the domestic supply. The Government of India (GOI) had launched the "National Biodiesel Mission (NBM)" after identifying *Jatropha curcas* as the most suitable tree-borne oilseed for biodiesel production (USDA-FAS 2012).

Jatropha oil has been used in India for several decades as biodiesel for the diesel fuel requirements of remote rural and forest communities; *Jatropha* oil can be used directly after extraction (i.e., without refining) in diesel generators and engines. Increased *Jatropha* oil production delivers economic benefits to India on the macroeconomic or national level as it reduces the nation's fossil fuel import bill for diesel production, minimizing the expenditure of India's foreign currency reserves for fuel, allowing India to increase its growing foreign currency reserves. And since *Jatropha* oil is carbon neutral, large-scale production will improve the country's carbon emissions profile. Finally, since no food-producing farmland is required for producing this biofuel, it is considered the most politically and morally acceptable choice among India's current biofuel options; it has no known negative impact on the production of the massive amounts of grains and other vital agriculture goods. India produces to meet the food requirements of its large population. Other biofuels which displace food crops from viable agricultural land such as corn ethanol or palm biodiesel have caused serious price increases for basic food grains and edible oils in other countries. It is also estimated that the potential availability of nonedible oils in India amounts to about 1 million tons per year. The most abundant resources are sal oil (180,000 tons), mahua oil (180,000 tons), neem oil (100,000 tons), and karanja oil (55,000 tons). However, based on extensive research carried out by various institutions in the country, the government identified *Jatropha curcas* oilseed as the major feedstock for biodiesel in India (Gonsalves 2006).

In India, ethanol is produced by the fermentation of molasses, a by-product of the sugar industry. India has 330 distilleries which can produce over 4 billion liters of rectified spirit (alcohol) per year in addition to 1.5 billion liters of fuel ethanol. Of this total, about 140 have the capacity to distill around 2 billion liters (USDA-FAS 2012) of conventional ethanol per year and could meet the demand for 5 % blending with gasoline.

Biofuel program in the country is at a nascent stage. Ethanol is produced in India from sugarcane molasses for blending with gasoline. Beginning January 2003, the Government of India mandated a 5 % ethanol blend in gasoline through its ambitious "Ethanol Blending Program (EBP)". Ethanol and alcohol production in India depends largely on availability of sugar molasses (a by-product of sugar production). Since sugarcane production in India is cyclical, ethanol production also varies with sugar and sugarcane production and therefore does not assure optimum supply levels needed to meet the demand at any given time (USDA-FAS 2012).

Since early 2001, the Ministry of Rural Development and several state governments have carried out programs to encourage large-scale planting of Jatropha on wastelands. States with the largest potential include Madhya Pradesh, Chhattisgarh, Rajasthan, Maharashtra, Andhra Pradesh, and Gujarat. The Planning Commission set an ambitious target of 11 million ha of *Jatropha* to be planted by 2012 in order to generate sufficient biodiesel to blend at 20 % with petro-diesel. However, the total *Jatropha* plantation area in the country is currently estimated at approximately 450,000 hectare (ha), of which about 60–70 % are new plantations and not yet into full production. The new *Jatropha* plantations are expected to come into maturity in the next 3–4 years (USDA-FAS 2009).

2.7 Other Renewable Energy Technologies

Solar thermal technologies, particularly solar water heating system, solar cookers, and solar generation systems are the most commercialized technologies among renewable energy technologies in India. Policies are set to provide further impetus to dissemination of solar technologies.

Biogas represents an alternative source of energy, derived mainly from organic wastes. In India, the use of biogas derived from animal waste, primarily cow dung, has been promoted for over three decades now. Biogas production is a clean low carbon technology for efficient management and conversion of organic wastes into clean renewable biogas and organic fertilizer source. It has the potential for leveraging sustainable livelihood development as well as tackling local (land, air, and water) and global pollution. Biogas obtained by anaerobic digestion of cattle dung and other loose and leafy organic matters/wastes can be used as an energy source for cooking, lighting, and other applications like refrigeration, electricity generation, and transport applications. Since biogas plants contribute in the reduction of greenhouse gases (GHG), hence they can be installed for availing of Clean Development Mechanism (CDM) benefits thereby generating additional revenue for wider coverage and reducing cost of biogas plants to government and beneficiaries. Based on the availability of cattle dung, an estimated potential of about 12 million family type biogas plants exists in the country, which can generate annually on an average basis about 15,000 million cubic meter of biogas. In addition, biogas plants also provide high-quality organic manure with soil nutrients which improves its fertility required for sustainable production and productivity. The "National Biogas and Manure Management Programme (NBMMP)" is being implemented in the country since 1981–1982 for promotion of biogas plants based on cattle dung and other organic wastes. The NBMMP mainly caters to setting up of family type biogas plants for meeting the cooking energy needs in rural areas of the country along with making enriched bio-fertilizer available to farmers. The availability of clean energy mitigates drudgery of rural women, reduces pressure on forests, and accentuates social benefits. In order to provide training support and technical backup, 12 Biogas Development and Training Centers (BDTCs) have been set up in Universities, Indian Institute of Technology (IITs), and other Technical Institutes. With the installation of 4.31 million family type biogas plants by January 2011, about 35 % of the estimated potential has been realized so far (MNRE).

Hydrogen has significant potential as a clean energy source for a broad range of applications including power production and transportation. Hydrogen can be used for power generation and also for transport applications. It is possible to use hydrogen in internal combustion (IC) engines, directly or mixed with diesel and compressed natural gas (CNG), or hydrogen can also be used directly as a fuel in fuel cells to produce electricity. Hydrogen energy is often mentioned as a potential solution for several challenges that the global energy system is facing. The advantages are the fact that hydrogen use results in nearly zero emissions at end use and that hydrogen opens up the possibility of decentralized production on the basis of a variety of fuels. But it is also found that hydrogen will not play a major role in India without considerable research, technology innovations, and cost reductions, mainly in fuel cell technology.

Hydrogen energy research in India started in 1976 on the initiative of the Government of India and covers almost all areas of technical relevance to the deployment of hydrogen as an energy vector (Sastri 1989). Now, hydrogen energy is also at an early stage of development. MNRE also funded research projects on different aspects of hydrogen energy technology development. India is the member of the International Partnership on Hydrogen Economy (IPHE) set up in Washington, D.C., in November 2003. Future challenges to India include lowering cost of hydrogen substantially, improving production rates from different methods, development of compact and inexpensive storage capacity, establishment of hydrogen network, development of hydrogen-fuelled integrated circuit engine, and efficiency improvement of different types of fuel cell systems. The road map envisages taking up of research, development, and demonstration activities in various sectors of hydrogen energy technologies and visualized goals of one million hydrogen-fuelled vehicles and 1,000 MW aggregate hydrogen-based power generation capacity to be set up in the country by 2020 (Ghosh et al. 2002).

The "National Hydrogen Energy Road Map (NHERM)" is a program in India initiated by the National hydrogen energy board (NHEB) in 2003 and approved in 2006 for bridging the technological gaps in different areas of hydrogen energy, including its production, storage, transportation and delivery, applications, safety codes and standards, and capacity building for the period up to 2020 (WHEC 2008). The Banaras Hindu University (BHU), Varanasi; Murugappa Chettiar Research Centre (MCRC), Chennai; and Indian Institute of Technology (IIT), Kharagpur are among the leading research groups working on biological, biomass, and other renewable energy routes to produce hydrogen. With R&D support from the MNRE, the MCRC has demonstrated hydrogen production in batch scale from distillery waste. The pilot plant is able to produce up to 18,000 l of hydrogen per hour (EAI 2013).

3 Act and Policies Pertaining to Power Distribution and Tariffs

3.1 Impact of the Electricity Act 2003

Table 1 shows the current status of renewable energy in India. The passage of "The Electricity Act 2003" has strengthened the process of reform in the Indian power sector and has enabled competition in the Indian power sector in bulk as well as retail electricity supply, in phases. This act has benefitted industrial consumers, independent power producers (IPPs), private utilities, and power equipment providers.

Since the Act provides provisions for setting up of captive power plants, industrial consumers can now escape the inefficiencies of the State Electricity Boards (SEBs). It also permits merchant generating units to provide electricity directly to industrial consumers. This provision will expand generation and bridge the demand-supply gap (www.crisil.com).

IPPs will also benefit from this act, as they can now sell power directly to consumers without any compulsion to first go through the SEBs. Hence, this act will provide a conducive environment for private investment in power generation and will promote decentralized power generation and distribution.

3.2 Tariff Policy, 2006

The Tariff Policy announced in January 2006 has the following provisions (Lalwani and Singh 2010):

1. Pursuant to provisions of section 86 (1) (e) of the act, the appropriate commission shall fix a minimum percentage for purchase of energy from such sources taking into account availability of such resources in the region and its impact on retail tariffs.
2. It will take some time before nonconventional technologies can compete with conventional sources in terms of cost of electricity. Therefore, procurement by distribution companies shall be done at preferential tariffs determined by the appropriate commission.
3. Such procurement by distribution licensees for future requirements shall be done, as far as possible, through competitive bidding process under section 63 of the act within suppliers offering energy from same type of nonconventional sources.
4. The Central Commission should lay down guidelines within 3 months for pricing non-firm power, especially from nonconventional sources, to be followed in cases where such procurement is not through competitive bidding.

4 Conclusion

Energy security, economic growth, and environment protection are the national energy policy drivers of any country. The need to boost the efforts for further development and promotion of renewable energy sources has been felt world over in light of high prices of crude oil. There is an urgent need for transition from petroleum-based energy systems to one based on renewable resources to decrease reliance on depleting reserves of fossil fuels and to mitigate climate change. In addition, renewable energy has the potential to create many employment opportunities at all levels, especially in rural areas. So isolated systems, whose cost depends on load factor, need to be linked with rural industry. Innovative financing is also a requirement.

According to the National Action Plan on Climate Change (NAPCC), other sources of renewable energy would be promoted. Specific action points that have been mentioned include promoting deployment, innovation, and basic research in renewable energy technologies; resolving the barriers to development and commercial deployment of biomass, hydropower, solar, and wind technologies; promoting straight biomass combustion and biomass gasification technologies; promoting the development and manufacture of small wind electric generators; and enhancing the regulatory/tariff regime in order to mainstream renewable energy sources in the national power system. Accordingly, increased focus is being laid on the deployment of renewable power that is likely to account for around 5 % in the electricity mix by 2032.

An emphasis should be given on presenting the real picture of massive renewable energy potential; it would be possible to attract foreign investments to herald a green energy revolution in India. India's quest for energy security and sustainable development rests a great deal on the ability to tap energy from alternate sources. Alternate fuels, essentially biofuels, are proposed to be progressively used for blending with diesel and petrol, mainly for transport applications. Finally, renewable energy provides enormous benefits and can contribute significantly in the national energy mix, and it is expected that the share of renewable energy in the total generation capacity will increase in the future.

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The Sahara Solar Breeder (SSB) Project Contributes to Global Sustainable Energy Production and Resource Conservation: An Overview

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1 Introduction

As we approach 2050 (ten billion persons on Earth), physical and environmental constraints in our use of energy resources are beginning to manifest themselves. Energy resources, increasingly scarce, are integral parts of the heritage of humanity. They require us to implement policies and strategies adapted to sustainable development because of the following reasons:

- Energy security, economic growth and environmental protection are the national energy policy drivers of any country of the world.
- Energy is central to achieving the interrelated economic, social and environmental aims of sustainable human development.
- Water is a fundamental part of our lives. It is a free and renewable natural resource. Human survival is dependent on water which has been ranked by experts as second only to oxygen as essential for life.
- Energy and water resources are intricately connected; we use energy to help us treat, clean and transport the water we need, and we use water to help us produce the energy we need.

According to the estimation done by the International Energy Agency (IEA), a 53 % increase in global energy consumption is foreseen by 2030; energy security is becoming a serious issue as fossil fuels are non-renewable energies (RE) and will deplete eventually in the near future. In addition, the world's oil resources will peak within a few decades to come (80 years at the most as depicted in Fig. 1) (ExxonMobil 2012), and in the search for other sustainable alternatives to mitigate some political, economic and environmental issues currently associated with the heavy reliance on fossil fuel, it is inevitable that the world is heading towards RE and new energy economy to reach sustainability (Fig. 2) by promoting clean energy technologies, pursuing energy efficiency and developing RE forms which are three orders of magnitude larger than current global energy use (Boudghene Stambouli et al. 2012). Moreover, climate changes, manifested in various forms, threaten the fragile balance of our ecosystem and require us to implement policies and strategies adapted to sustainable development. For this reason, all countries should seek technologies from all disciplines applicable in RE and energy efficiency (EE). Indeed, considering them together, by improving access to clean and green modern energy, offers substantial economic and environmental benefits for the world as well as a unique opportunity for technological advancement in the energy fields. The idea of valorisation of energy

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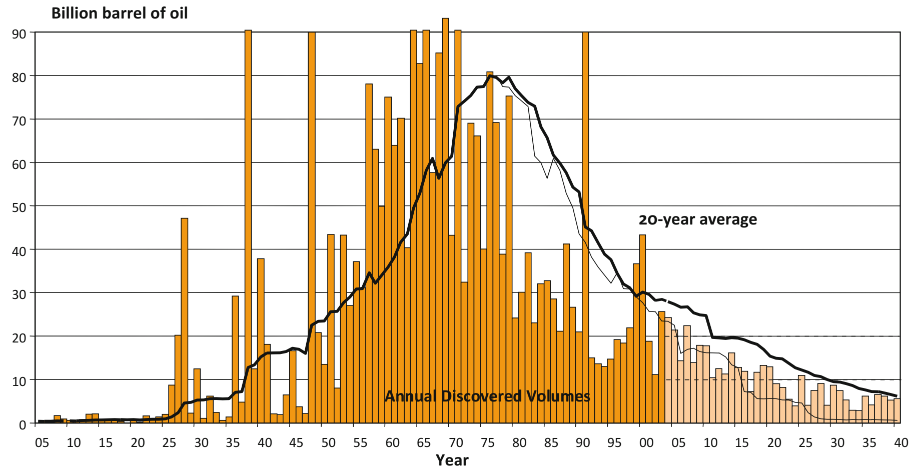


Fig. 1 Confronting the supply challenges, discovered annual volume of oil (1905–2040)

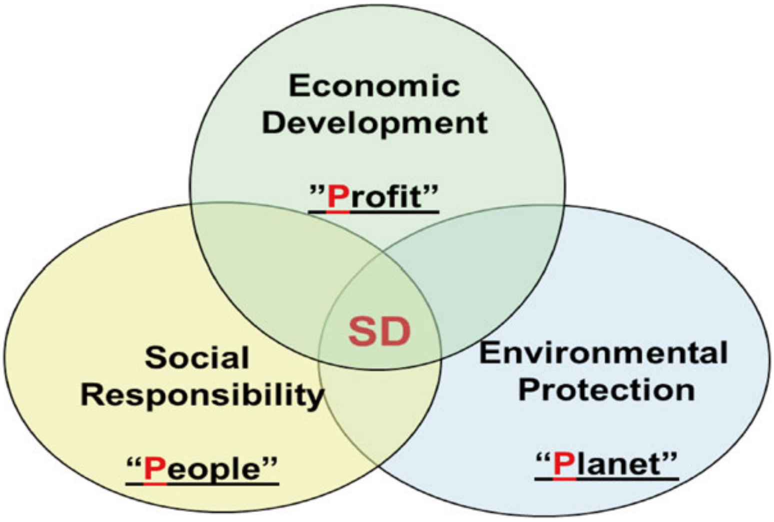


Fig. 2 Elements for sustainable development

resources for innovation is to give a vision about the future, take into account the experience of the energy sectors during the last decade, about the development of the RE, focused from a double perspective in terms of regulative and technological.

Depletion of oil resources can be considered both as a natural and technological risk. This is a serious risk that deserves all the attention and efforts of the scientific community, responsible not only to think about alternatives when there will be no oil but to find solutions for a more serene economy free from this threat.

Solar energy is the most promising source of clean, renewable energy, and it has the greatest potential of any power source to solve the world's energy problems (Kadir and Rafeeu 2010). The energy from sunlight is huge, 0.01 % utilisation would cover the entire world. This offers a unique opportunity of technological advancing in the solar energy field. Indeed, improved access to clean and green modern energy is a fundamental step to poverty reduction; with advanced and technical knowledge and resources, industrialised and developed nations can facilitate the

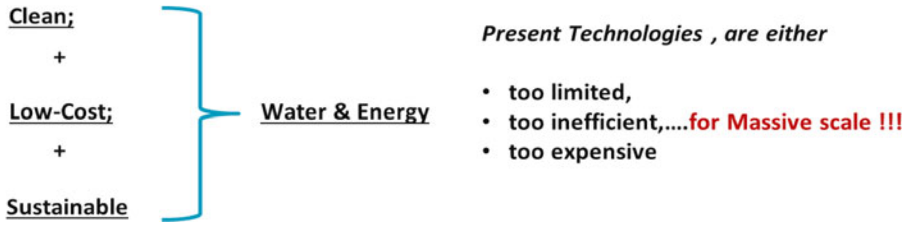


Fig. 3 Emerging issues need to be addressed in the society

achievement of sustainable development for the whole world.

Clean and secure energy is the key to a sustainable civilisation, to human security, to a reliable energy supply, to climate stability and biodiversity for global and intergenerational justice and to more civilisation and wealth. Energy is also the driving force of life, usually accompanying conversion from one form to another including heat, light, mechanical, electric and chemical energy as well as changes in mass. Without energy flow, no living thing can survive. Throughout human history energy use (and the rate of consumption) and the advancement of civilisations have increased simultaneously. By considering the Sahara Solar Breeder (SSB) project, developed in this article, we can easily consider it as, first, being an energy/climate security with global justice and development of civilisation for the whole world and, second, a clever global development strategy for solving energy and climate problems with existing solar-grade silicon (Si) production from sand technology for a sustainable world. SSB may be a great solution towards world sustainability since it proposes a plan to overcome issues the society is facing today as shown in Fig. 3 and summarised as follows:

- Climate change (mostly from burning of fossil fuel)
- Increase in the prices of limited energy resources
- Increase in the prices of energy-intensive commodities like fertiliser
- Air and water pollution
- Economic development in all aspects
- National security
- Poverty and global health
- How to keep society free of geopolitical conflict

2 The Sahara Solar Breeder (SSB) Project: A New Global Climate Policy

On Earth, there are three kinds of energy resources available: (1) geothermal and nuclear (radioactive) energy, (2) fossil fuels (crude oil, natural gas and coal) and (3) renewable energy from incident sunlight, water (hydropower), wind and biomass. Energy is the engine of the universe, engine of all the fundamental processes of nature on Earth and engine of the world economy. The world is full of energy resources, but their supply is finite. Our response is to find ways to use these resources more efficiently and develop new sources for energy. Energy needs to be converted into power in order to make use of it. The different forms of energy are summarised as being fossil, fissile, renewable and new energies which can be presented as mechanical, chemical, electrical, nuclear and thermal energies. Energy researchers should creatively apply their knowledge of science (Physics and Chemistry), engineering (electrical and mechanical) and economics to confront the global challenges of energy supply and demand (Energy Engineering 2013). Figure 4 shows how we need science and technology that are poised to play a transformative role in providing clean and sustainable energy. The SSB project may be a solution since it proposes a plan of international partnership in basic research and development, industrial production, trade and finance to construct, gradually over the coming decades, a ‘Global Clean Energy Superhighway’ starting in the Sahara desert in North Africa beginning from Algeria.

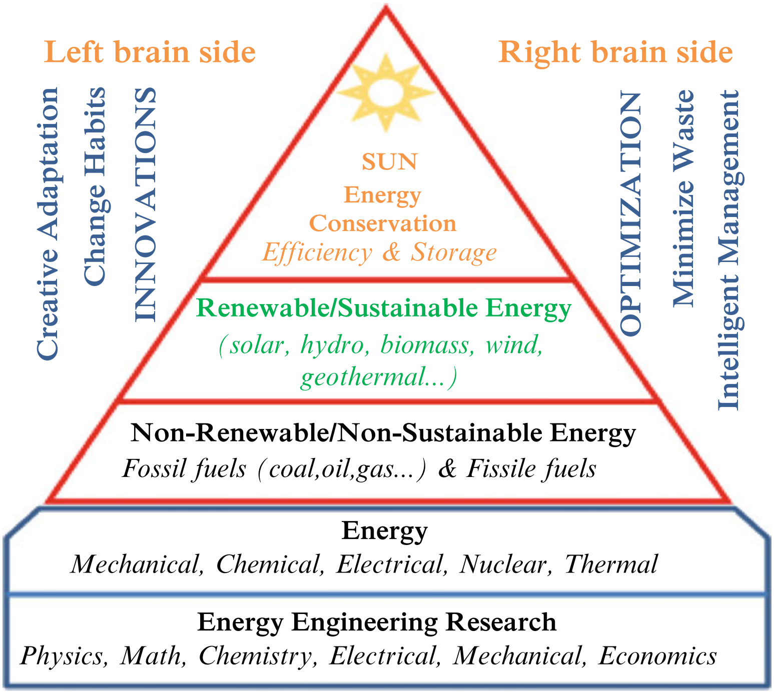


Fig. 4 Thrust areas of energy engineering, forms of energy and energy pyramid

Both nuclear and fossil fuels will run out in a few hundred years, at the latest. Nuclear power stations are questionable as a main energy source since their vulnerability and serious hazards were realised following the Fukushima Daiichi nuclear disaster on March 11, 2011. Carbon dioxide (CO_2) as the main product of fossil fuel combustion is a major contributor to climate change. Therefore, we are reaching the point where we must think about alternative energy for the future. The Middle East and North African (MENA) countries, which are presently enjoying economic prosperity from oil and gas production, recognise the situation and have started thinking about future energy strategies. Recognising that a desert is filled with sand, the most abundant natural resource on Earth, and that the sand could be of value by chemically converting it into solar silicon, the SSB project proposed by the Science Council of Japan (Koinuma et al. 2009, 2010; Kurokawa et al. 2009; Boudghene Stambouli and Koinuma 2012) is one option that is attracting much MENA attention, partly because of the possibility of using desert sands for photovoltaic (PV) power generation to supply the energy needs of the world. There are two renowned big benefits of Sahara and other deserts from the viewpoint of energy utilisation, i.e. vast land and ample sunshine, which attract much interest for harvesting new clean energy from deserts. Therefore, material and energy are the key components of our life and indispensable for the sustainable development of our world.

The SSB project involves building manufacturing plants around the Sahara desert that would extract silica from the sand and turn it into solar panels to generate renewable energy. The renewable energy from the first facility will then be used to breed more manufacturing plants and, in turn, more solar panels to generate ever increasing amounts of solar power. The ultimate goal is to build enough plants until the breeding strategy can deliver 100 GW of electricity to provide 50 % of the world's electrical power generation capacity by 2050 which would be delivered via a global superconducting electrical grid to turn the world's biggest desert into the world's biggest power station, taking advantage of two resources that are

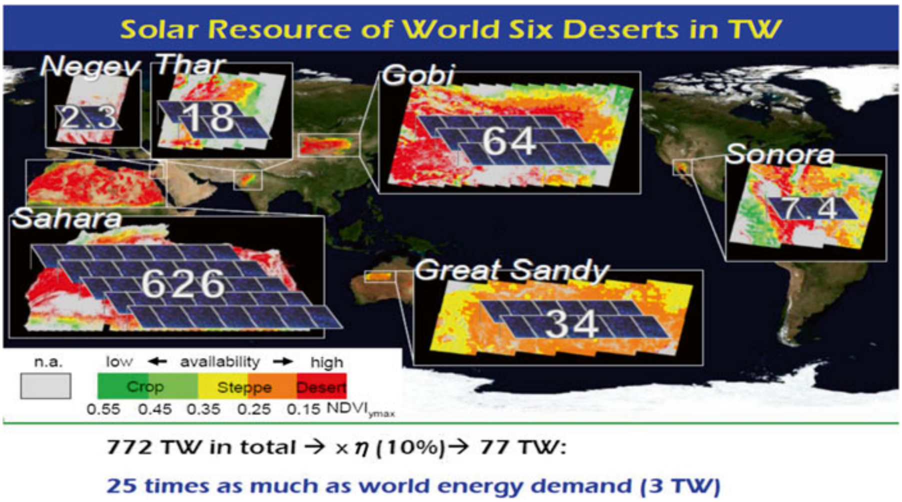


Fig. 5 Solar resources of the world's six deserts (in TW)

found in abundance in the Sahara, namely, silica and sunlight (Fig. 5) (Kurokawa et al. 2009).

The strategic objective of SSB is the establishment of a 'Global Clean Energy Superhighway' as the solution to global energy challenges, water shortages, levelling of electric power supply in the world, climate change and other environmental problems arising from the current fossil fuel heavy global energy paradigm. The development and realisation of the SSB project, in the Sahara of Algeria which covers a total area of 2,048,297 km^2, approximately 86 % of the total area of the country, will tackle the key challenges and issues related to the field of PV putting forward the material to research and development perspective and promoting innovative processes for solar-grade Si with a focus on the utilisation of Sahara sands.

Although solar power-producing devices have been around for over 50 years, solar electricity devices often referred to as PV are still considered as a cutting-edge technology. Globally, there are about 1,700 TW of solar power theoretically available over land for PV. The capture of even 1 % of this power would supply more than the world's power needs. The cumulative installed solar PV power at the end of 2007 was 8.7 GW, with less than 1 GW in the form of PV power stations, and most of them rest on rooftops (Jacobson 2009). The capacity factor of solar PV ranges from 0.1 to 0.2, depending on location, cloudiness, panel tilt and efficiency of the panel. Current technology of PV capacity factors rarely exceed 0.2 based on calculations that account for many factors, including solar cell temperature, conversion losses and solar isolation (NREL 2008). PV systems have a number of merits and unique advantages over conventional power-generating technologies. PV systems can be designed for a variety of applications and operational requirements and can be used for either centralised or distributed power generation. Energy independence and environmental compatibility are two attractive features of PV systems. The fuel (sunlight) is free and no noise or pollution is created from operating PV systems. In general, PV systems that are well designed and properly installed require minimal maintenance and have long service lifetimes. However, at present, the high cost of PV modules and equipment are the primary limiting factor for the technology.

SSB will, therefore, help to migrate from the unsustainable currently excessive fossil fuel-based global energy paradigm to a more sustainable one. It will also help to meet global energy challenges and mitigate climate change and other environmental problems. SSB is more than an energy solution. It is an integrated community, socio-economic, industrial, agricultural, environmental and science and technology development solution. In particular, through desalination and adequate irrigation

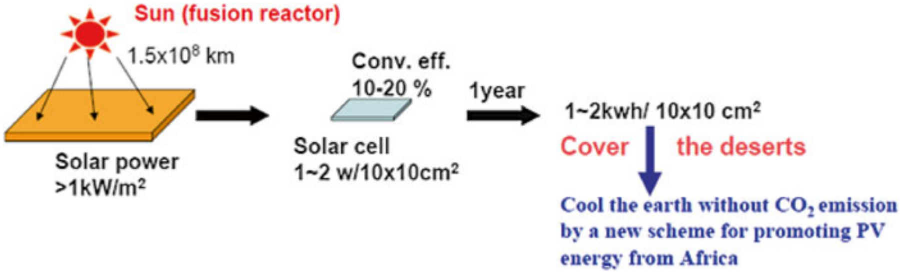


Fig. 6 Photovoltaic system using the photoelectric effect of turning light energy into electricity

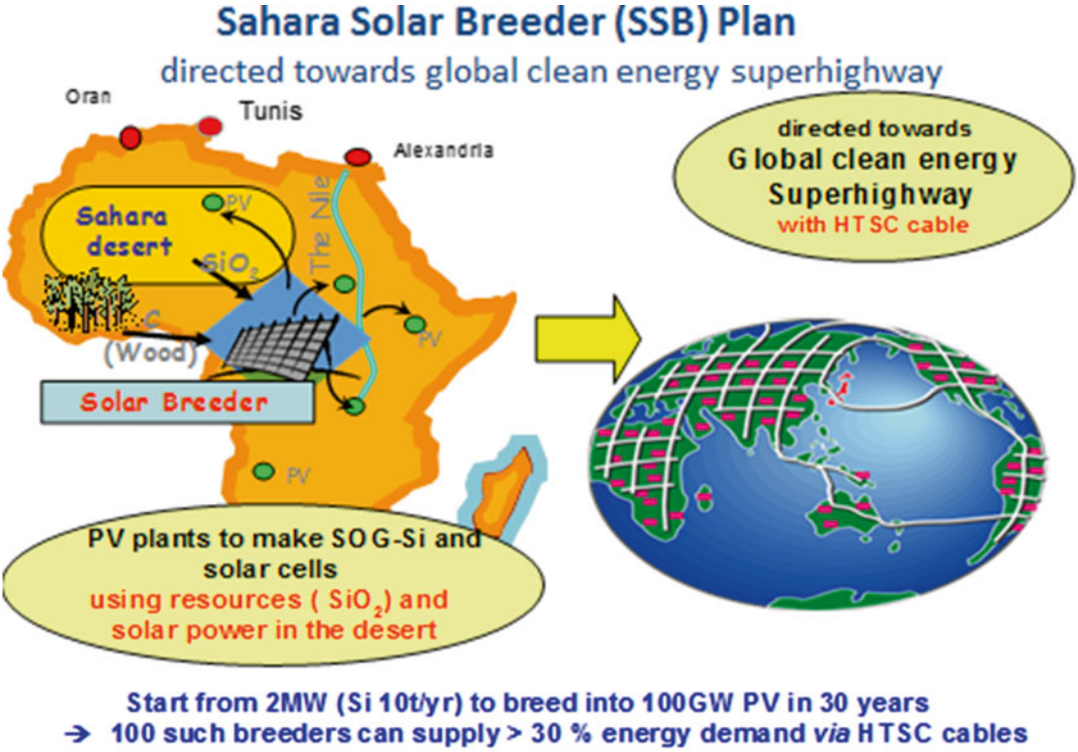


Fig. 7 Concept, plan and framework of SSB

technologies, it will help mainstream marginal desert water resources and lands back into national, regional and international development processes. The concept of the SSB is depicted in Figs. 6 and 7 (Koinuma et al. 2009; Boudghene Stambouli and Koinuma 2012). Scenarios can create a vision for the future and guide decision makers.

The SSB project serves as the initiative for the super Apollo project (the Sahara Solar Breeder Foundation; http://www.ssb-foundation.com) to provide the paradigm shift in global energy system. SSB merits and difference from other projects is that:

- High-skill jobs are created continuously.
  - People enjoy to study and work over generations.
- Deserts are converted to area for more life.
  - Happy to manage growth of own economy
- Sustainable distribution of energy to the globe.
  - Supporting the world's sustainability (social responsibility)

3 The Sahara Solar Breeder (SSB) Project

3.1 Goals of SSB

The goals of SSB towards sustainable development are founded on four pillars:

- Starting from basic research and development
- Coupling PV with high-critical-temperature superconducting cables (HT_cSC) for clean energy generation and energy-saving transmission
- Science and technology education and training for African people
- Solving global crisis by international cooperation and policy

3.2 Strategies of SSB

The three basic strategies of the Sahara Solar Energy Research Center (SSERC) programmes and indicative targets are based on the following items:

- Innovation processes for solar silicon with a focus on using Sahara sands; the following objectives have been selected:
  - Purification of silica sand by a thermodynamics-based process designed in Tokyo and Nihon universities
  - Reduction of silica sand by carbothermal (Hirosaki University) and plasma (National Institute for Materials Science, NIMS) processes
  - Technology transfer to University of Science and Technology of Oron (USTO)
- Quantitative data collection for installation of PV and HT_cSC systems: the following objectives have been selected:
  - PV system installation at Saida site
  - Data collection for DC power transmission through desert environment
- Education and training on energy problems and sustainable development; the following objectives have been selected:
  - Installation of WebEl system at USTO and Saida universities
  - Development of PV materials, devices and system
  - PV application such as desalination, green innovation in desert
  - Personal movement between Algeria and Japan

4 Components of the Sahara Solar Breeder (SSB) Project

The most important objectives of SSB's energy policy and its portfolio include five basic strategies:

- Basic, applied, practical research and development in Japan, North Africa, the Middle East, Africa and other regions of the world
- Industrial production of silicon from sand
- Industrial production of cells, modules, panels and other PV devices
- Building, operating, networking and monitoring very large scale photovoltaic power stations (VLS-PVPS)
- Environment monitoring and gradual implementation of SSB

One of the strengths of PV is found in its decentralised applications. This is particularly true for supplying isolated consumers in areas of low population density where the demand consists essentially of satisfying basic energy requirements. Other notable characteristics of PV are:

- Modular design enabling it to be extended according to need
- The possibility of developing small businesses in areas of low economic development
- Protection of the environment
- Limited capital assets capable of being used flexibly and in a decentralised way and of being moved about over longer periods of time

The developing strategy, by the SSB project, has been elaborated to promote the dissemination of renewable energies on sites where they are profitable compared to classical energies and to guide scientific research efforts in order to allow generalisation of renewable energy via mass production. The aims to be achieved consist of the contribution to a conservative policy for hydrocarbons both by increasing the renewable energy share within the international energy balance and by improving the living conditions of isolated communities. The first operation of installation of PV plant in Saida town, considered to be the gate of the Algerian Sahara, would allow on one hand the electricity supply and on the other hand to collect information about:

- Equipment behaviour in Saharan environment
- Matching the systems with the electricity supply
- Maintenance of organisation and management
- Technical-economic system optimisation

SSB will then ensure an energy/climate security with global justice and development of civilisation for the whole world, a clever global development strategy for solving the energy and climate problems with existing solar-grade Si production from Sahara sand technology for a sustainable world. Sahara has plenty of silica as primary material for Si and sunlight as solar energy source. Table 1 summarises the power generation capability (evaluated from material needs, efficiency and natural resources) of different solar cell materials. Moreover, SSB is an integrated community, socio-economic, industrial, agricultural, environmental and science and technology development solution. In particular, through desalination and adequate irrigation technologies, it will help mainstream marginal desert water resources and lands back into national, regional and international development processes as shown in Fig. 8 (Boudghene Stambouli and Koinuma 2012). Scenarios help us understand the limitations of our ‘mental maps’ of the world – to think the unthinkable, anticipate the unknowable and utilise both to make better strategic decisions.

5 Innovative Silicon Technologies from the Sahara Solar Breeder Project

Material and energy are key components of our life and indispensable for sustainable development of our world. There are two renowned big benefits of Sahara and other deserts from the viewpoint of energy utilisation, i.e. vast land and ample sunshine, which attract much interest for harvesting new clean energy from deserts. The SSB project we proposed and started initiative R&D with Algeria is aiming energy harvest from

Table 1 The power generation capability of different solar cell materials

Materials	Type	Thickness (μm)	Conversion efficiency (%)	Element	Wp/g	Resource (10^3 t)	Capability of power generation
c-Si	W	200	20	Si	0.1	∞	∞ (GW)
	TF	20	15	Si	0.75	∞	∞ (GW)
A-Si	TF	0.7	10	Si(H)	19	∞	∞ (GW)
InP	W	200	25	In	0.33	1.68	0.56 (GW)
	TF	2	20	In	26	1.68	44.5 (GW)
GaAs	W	200	25	Ga(As)	0.49	110	53.9 (GW)
	TF	2	20	Ga(As)	39	110	4,310 (GW)
$CuInSe_2$	TF	2	15	In (Cu)	38	1.63	64 (GW)
				Se	28	83	2,290 (GW)
CdTe	TF	2	15	Cd	27	555	15,100 (GW)
				Te	24	22	526 (GW)
Ge	W	200	15	Ge	0.14	44	0.62 (GW)
	TF	0.5	15	Ge	60	44	250 (GW)

W wafer, *TF* thin film

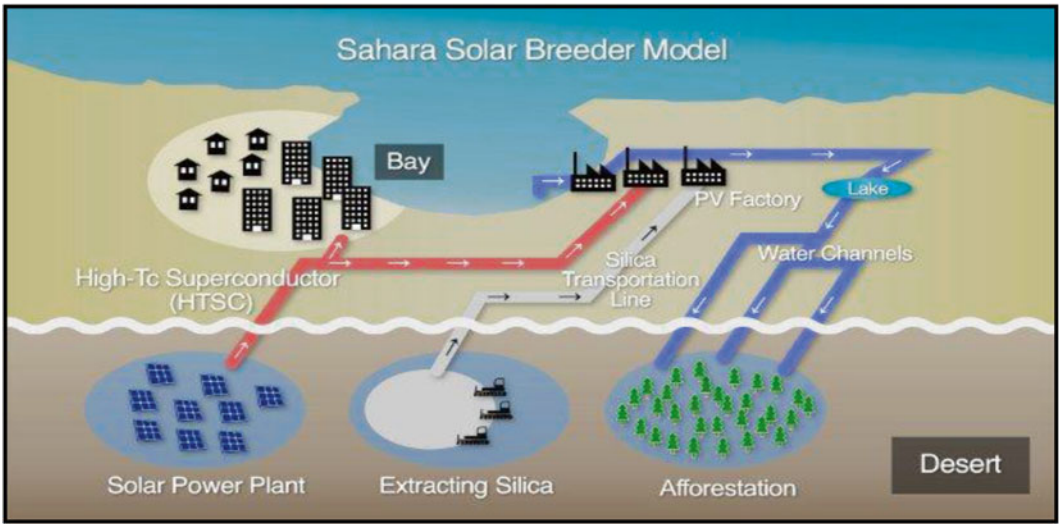


Fig. 8 SSB model: key to a sustainable civilisation

the desert by extracting the extra value from the sand through the cooperative research with Saharan countries. We have been working for proving the feasibility and superiority of SSB-PV to provide an absolute solution of sustainable development for the future world (Koinuma and Boudghene Stambouli 2013). The first key technological task is the development of solar-grade Si from desert sand.

5.1 General Scheme of Silicon Process

Silicon dioxide (SiO_2) is the most abundant natural resource on Earth. If we could develop an efficient process for reducing SiO_2 to solar-cell-grade silicon (purity level >99.9999 %) and use this to make polycrystalline (polysilicon) solar cells, solar energy could be converted into electricity with about 15 % efficiency. This efficiency appears to be lower than conventional power generation systems (about 40 % efficient), but it is substantially higher in view of the fact that sunlight is fuel free.

Process flow of silicon is depicted in Fig. 8. Silicon is the second most abundant element in the Earth's crust, much more than iron (Fe), but Si production scale is far lower than Fe, especially of semiconductor grade (SEG-) by four to five orders of magnitude. The over-specified quality (>10 nines) of SEG-Si had been the main reason of the high cost of Si solar cell. Despite the recent temporal low price of Si by the overproduction in China, the development of new SOG-Si (~6 nines purity) process with minimum energy consumption is presumed to be the first priority for realising clean sustainable energy for the future. It must be noted that SOG-Si PV is more promising and not only fashionable, i.e. so-called innovative and next-generation PV, but also conventional power stations (Koinuma et al. 2013). Si renaissance strategy is derived from a pioneering but forgotten research in the 1980s on SOG-Si from silica sands (Koinuma et al. 2013) to make it smarter by the 'Beyond Siemens process' shown in Figs. 9 and 10. Among various PV systems, only Si PV (4 % of desert areas) could cover all the energy needs in the world.

5.2 Purification of Silica Sands

The collaborative industry (Taiheiyo Cement Co. Ltd., in Japan) developed a process for purifying amorphous silica sand in Japan by the original wet process of dissolution in aqueous NaOH solution followed by the neutralisation to

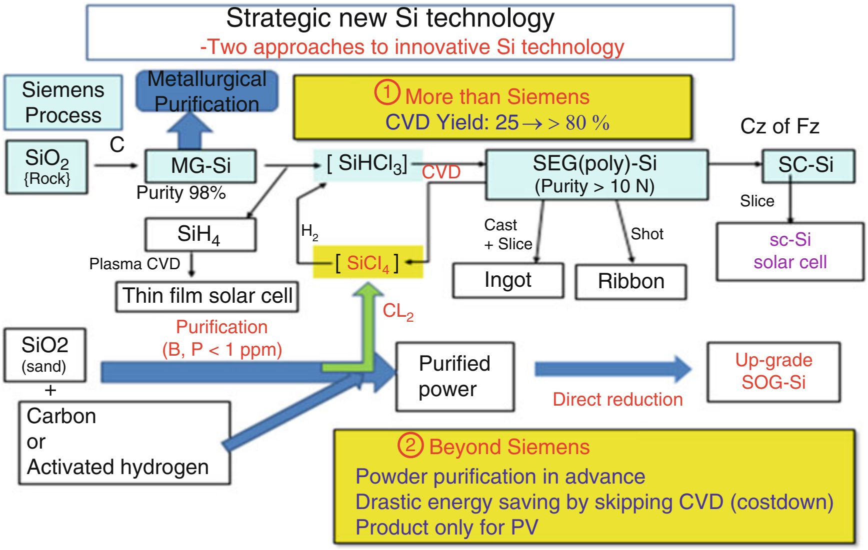


Fig. 9 Strategic new Si technology including two innovative approaches

precipitate SiO_2. This process could not be applied to desert sand directly due to low solubility of the sand which is composed primarily of crystalline silica. We found that this problem could be solved by applying the ball milling to the sand which had been gravimetrically separated and rinsed with HCl in advance. Thus, we could merge the purification process of desert sand with that of amorphous silica as shown in Fig. 11. ICP-OES analysis verified sub-ppm levels of boron (B) and phosphorus (P) impurities in the silica purified from Sahara sand at Hassi Messaoud location in Algeria. Metallic impurities are known to be segregated effectively by the unidirectional solidification process.

5.3 Direct Reduction of Purified Silica to Silicon

In the 1980s, NEDO-supported Nippon sheet glass/JFE Co. joint project, which reported that purified silica reduction with carbon powder in an arc furnace using graphite shafts to feed pelleted SiO_2 and C to give 6 nine grade SOG-Si. This Si was used to fabricate polycrystalline Si solar cells. Energy conversion efficiencies higher than 12 % was achieved (NEDO 1987). It must be noted that this efficiency is higher than the efficiencies of conventional thin-film solar cells. Hence, we can get Si solar cells with reasonably high efficiency and durability once we have SOG-Si. By omitting the energy-consuming CVD process (~100 kWh/kg-Si) in the conventional Siemens process (Figs. 9 and 10), drastic energy saving and production cost reduction should be achieved.

6 Conclusions and Recommendations

The importance of access to clean energy cannot be overstated. Research, development and demonstration (RD and D) are the foundation for progress and change towards sustainable energy systems throughout the world to protect the global life-supporting systems and reduce the risk of geopolitical conflicts over fossil fuel resources which are quickly depleting materials. We need more electricity from RE and more investment and initiative to make solar energy cost-effective and competitive. There are good examples of business initiatives to enhance

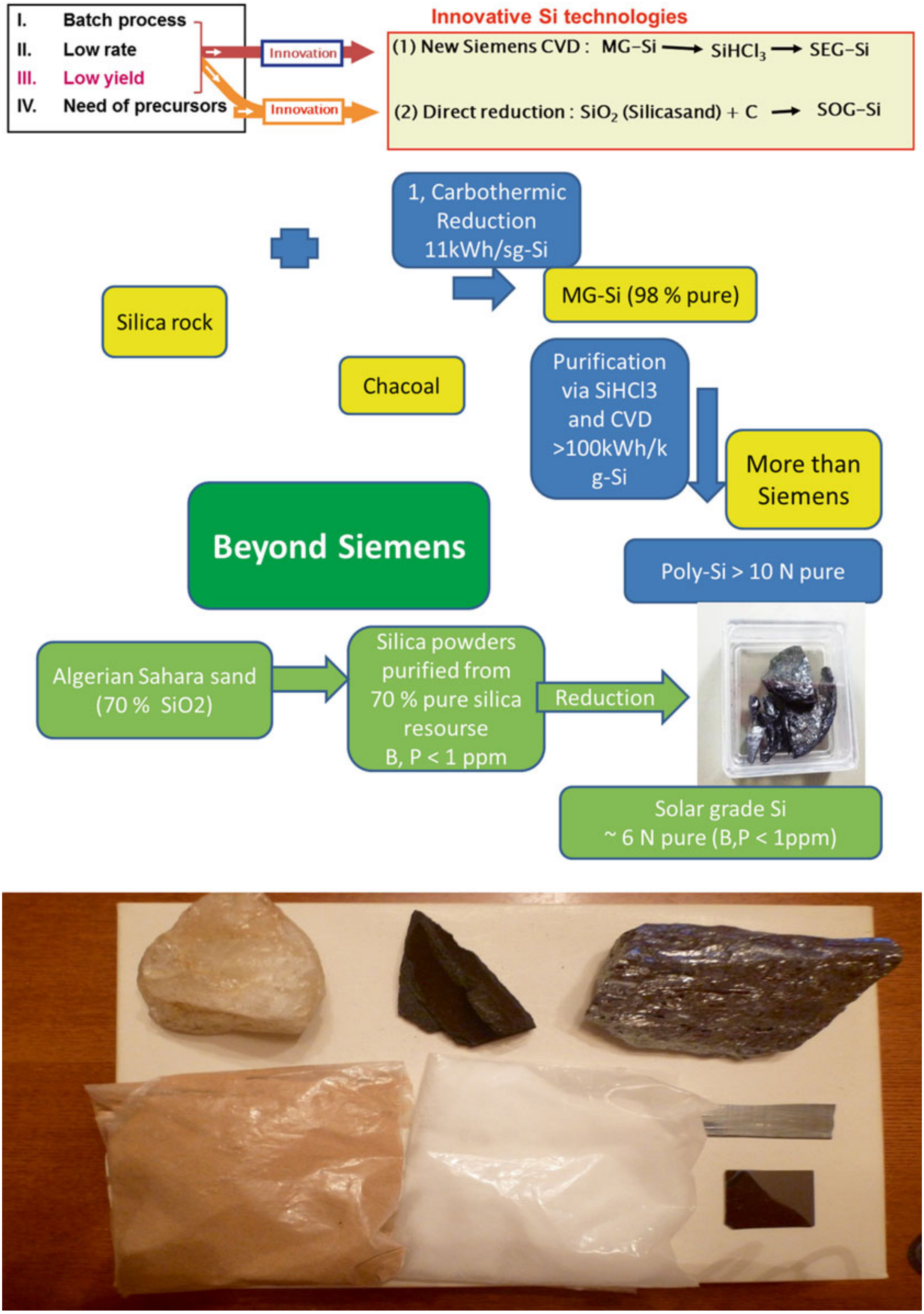
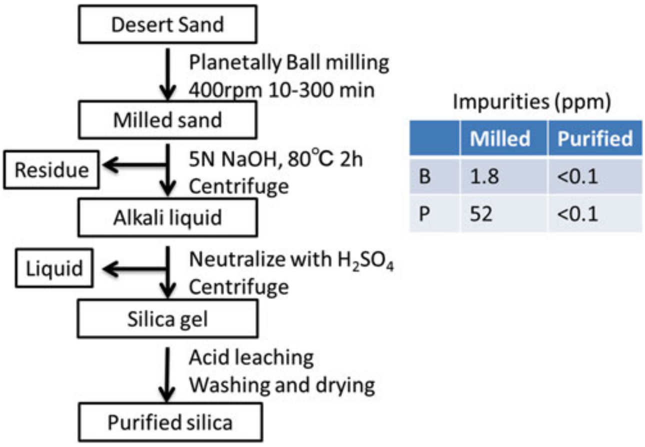


Fig. 10 Innovative Si technologies by 'Beyond Siemens process'



	Milled	Purified
B	1.8	<0.1
P	52	<0.1

Fig. 11 Process of sand purification and their impurities

energy security for communities in energy-stressed regions, and the lessons of good practice, from experienced countries, need to be more widely disseminated. Cooperation is crucial; problems cannot be solved by one country alone. We need to learn from each other at local, regional and global hierarchical levels; however, cooperation is crucial. The drivers of regional cooperation on water and energy are as follows:

- Geography
- Resources endowment/variability
- Trade
- Democracy and governance
- Power asymmetries
- Diplomatic relationship
- Political regimes
- Colonial heritages

Clean and secure energy:

- Key to a sustainable civilisation
- Key to human security
- Key to a reliable energy supply
- Key to climate stability and biodiversity
- Key for global and intergenerational justice
- Key to more civilisation and wealth

SSB:

- Energy/climate security with global justice and development of civilisation for the whole world
- Clever global development strategy for solving energy and climate problems with existing solar-grade silicon production from Sahara sand technology for a sustainable world

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Clean Development Mechanism: A Key to Sustainable Development

Himanshu Nautiyal and Varun

Abstract

The emission of high amount of greenhouse gases (GHG) into the atmosphere is the major cause for climate change. The average temperature of the Earth is increasing year by year. Kyoto Protocol was introduced to set strict rules to control the high level of GHG emissions and promote sustainable development. Clean Development Mechanism (CDM) is an effective mechanism to reduce emissions by promoting sustainable energy projects. It allows emissions trading among different countries and helps in achieving goals of emissions reduction. This chapter presents an overview of CDM, its methodology and impediments associated with it.

Keywords

Greenhouse gases • Kyoto Protocol • Certified emissions reductions

1 Introduction

Climate change is a part of the natural cycle of the Earth, but industrialization and other commercial activities have a significant role in the contribution of greenhouse gas (GHG) emissions into the atmosphere. The high amount of GHG emissions in the atmosphere leads to environmental devastation. Many major changes in natural climate have been experienced throughout the world. The primary consequence of climate variation is global warming which has become one of the most serious environmental concerns today because the Earth is getting warmer year by year. According to a study on the world's temperature variation conducted by NASA's Goddard Institute for Space Studies (GISS), the temperature of the Earth is increasing at the rate of 0.15–0.20 °C per decade. As a matter of fact, the release of high amounts of GHG emissions into the atmosphere is almost the main cause of global warming. These gases absorb and trap thermal radiations in the atmosphere which causes the average temperature of the Earth to increase. Power generation, transportation,

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Table 1 List of countries by 2012 emissions (PBL Netherlands Environmental Assessment Agency 2013)

S. No.	Country	Million tonnes of CO_2
1	China	9,900
2	The United States	5,200
3	India	1,970
4	Russian Federation	1,770
5	Japan	1,320
6	Germany	810
7	South Korea	640
8	Canada	560
9	The United Kingdom	490
10	Mexico	490

industrialization and agriculture sectors are valuable and splendid signs of development but are also the main sources of GHG emissions. All nations of the world are trying passionately to enhance their TIE (technology, industrialization and energy) for economic development. Although technology and industrialization are the wheels of development and are very essential, above all energy/power is the main requirement to drive the vehicle of development. Today almost all nations depend on fossil fuels and other petroleum product (having a fast-depleting nature and the main source of GHG emissions)-based power generations to fulfil their power requirement. Consequently, power generation contributes to the major portion of total global GHG emissions (US EPA). Table 1 shows the list of countries having major role in producing the world's total emissions (PBL Netherlands Environmental Assessment Agency 2013). According to the list, the largest producer of greenhouse emissions is China. After this the United States, India, Russian Federation and Japan are other major GHG contributors. Finally, a polluted and contaminated environment is the outcome of such economic development shown in Fig. 1.

Carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and some other fluorinated gases are the main constituents of GHG that contaminate the Earth's atmosphere. CO_2 is the main GHG produced by almost all human, commercial and industrial activities. CO_2 present in the atmosphere is part of the carbon cycle (i.e. relation among animals, plants, soil, etc., to exchange carbon), but the generation of more CO_2 from human and industrial activities disturbs the carbon cycle resulting in the alteration of atmospheric CO_2 level. It would not be wrong to say that every human activity, whether it is domestic or commercial, from pin to aircraft, is responsible for releasing some CO_2 into the atmosphere. According to the data presented by Scripps CO_2 Program at Mauna Loa Observatory, Hawaii, the CO_2 level in the atmosphere recorded in September 15, 2013, is 391.31 parts per million (ppm), but the troublesome thing is that the CO_2 level is increasing year by year at a startling rate (www.CO$_2$now.org). Figure 2 shows a serious picture of increase of CO_2 level in the atmosphere from 2004 to 2013. The chart revealed that CO_2 concentration is increased by 5.15 % in the last 10 years. Therefore, this troublesome situation of rapid boost in CO_2 level in the Earth's atmosphere is a big challenge for all to think about a sustainable environment in the future, and in addition this issue propels us to think about the control of GHG emissions which is unquestionably essential to make Earth a suitable place for all human beings.

In order to reduce GHG emissions, a framework was formed by the United Nations, viz. United Nations Framework Convention on Climate Change (UNFCCC) in 1992. UNFCCC was formed in a notable conference, viz. United Nations Conference on Environment and Development (UNCED) hosted by Brazil at Rio de Janeiro which is considered as an important Earth Summit. About 172 governments (with 108 at level of state or government) and many non-governmental organisations (NGOs) participated in the conference, and the main topic of the conference was environment and sustainable development. The main and important result that came from the UNCED summit was that there is an interrelation between many social, economic and environmental factors and environmental issues, economic matters and social justice issues. In addition to this, other appreciated outcomes of the UNCED were Rio Declaration, formation of UNFCCC, formation of Convention on Biological Diversity (CBD) and statement of forest principles (www.CO$_2$now.org).

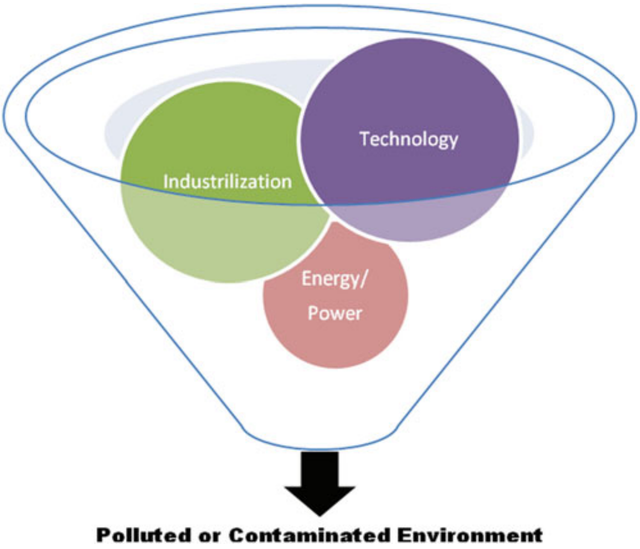

Fig. 1 Economic development with environmental devastation

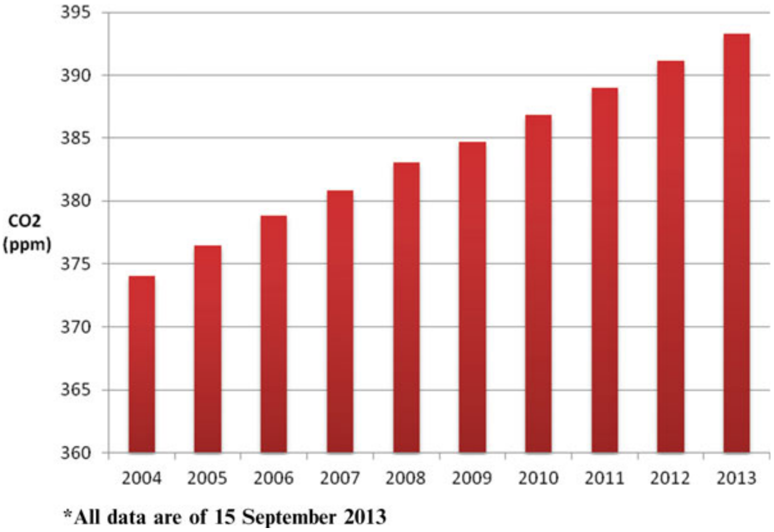

Fig. 2 Increase of CO_2 level in the atmosphere in the last 10 years (www.CO_2now.org)

UNFCCC was a global treaty whose main concern was to control the GHG emissions. Actually, it was an agreement signed by 154 governments in which critical issues on climate change were raised and many countries agreed to think on something to stabilise and reduce GHG emissions in order to control climate change issues.

Although 20 years ago from the UNCED summit conference was held on Stockholm (Sweden), viz. United Nations Conference on Human Environment, in 1972 to discuss the condition of global environment, UNCED was more notable and valuable in terms of environmental sustainability. Nevertheless, Stockholm conference was considered as an initiation of awareness of environmental problems at political and public level. The main objective of the Stockholm conference was to provide awareness of environmental degradation issues to the nations of the world.

In December 1997, an agreement was adopted in Kyoto city, Japan, popularly known as the Kyoto Protocol. It was a treaty that made some strict rules for all developed countries to reduce their emissions. There were some strict binding rules about emissions generation for those countries that are responsible for producing high GHG emissions into the atmosphere. The important thing that arouse from the Kyoto Protocols was the introduction of three flexible mechanisms, viz. emission trading, joint implementation and Clean Development Mechanism (CDM). Basically, CDM was an important mechanism established under Article 12 of the Kyoto Protocol. It was completely dedicated to climate change and other environmental problems. The most important objective of the Kyoto Protocol was the promotion of sustainable development in developing countries (non-Annex I countries) and control emissions in developed countries (Annex I countries).

2 CDM: An Overview

As discussed, emission trading, joint implementation and CDM were the three mechanisms included in the Kyoto Protocol. Among these three mechanisms, CDM may be really effective for developed countries to reduce their emissions. In addition to this, it is also beneficial for countries to promote sustainable development in developing countries (Philibert 2000). It is a well offsetting mechanism which promotes emissions reduction trading by promoting sustainable projects as well as projects having low emissions. Sustainable development in developing countries as an objective of CDM acts as a platform to increase the growth rate of developing countries. CDM has come out not only as a methodology but as a big market in which many projects have been registered in the world, out of which some have been established and some are in the implementing stage. Trading of emission credit known as certified emission reductions (CERs) is the main part that comes under CDM. All the advantages of CDM projects especially emissions reduction are expressed in CERs. It is all the matter of reduction GHG emissions and CO_2 is the primary constituent of GHG and that is why CERs are generally expressed in terms of "tonnes of CO_2" emissions reduction associated with a project. Also the other constituents of GHG discussed in the previous section (CH_4, N_2O, etc.) are also expressed in "tonnes of CO_2". In fact 1 tonne of CO_2 reduction corresponds to one CER. A developing country having projects with emissions reduction objectives can sell its CERs.

Proper promotion, implementation and supervision are essentially required for the effective development of CDM projects. Therefore, an executive panel properly known as the CDM Executive Board (EB) was formed especially for the supervision of CDM projects in the Conference of Parties (COP) 7 held in Marrakech, Morocco, from October 29 to November 10, 2001. The board comprised members of parties of the Kyoto Protocol. To execute various activities of promotion, implementation and supervision of CDM projects, EB gives authority to some organisations and firms known as designated operational entities (DOEs). In COP 7, Marrakech, certain norms and criteria were formed for CDM registration which are finally conceded in the COP held in New Delhi, India, from October 23 to November 1, 2002, and all the projects willing to register under CDM must follow the norms.

A CDM project follows a cycle from planning to implementation which is shown in Fig. 3. The first stage of this cycle is project development in which a document of project proposal which is known as project design document is prepared. The next step is project approval by the host country, and the designated national authority of the country issues a project approval letter (essentially required) to the owner. After this, project validation is done in which the project design document is verified by a DOE. After project validation the design document is eligible for registration process. So the design document is sent to the EB for registration. When the registration process is over, then the project monitoring is done in which the owner of the project checks and monitors the emissions reduction associated with the project and sends another to a DOE. A DOE audits the emissions reduction sent by the owner. If the claimed emissions reduction is found satisfactory, then a DOE approves and certifies the project. Finally, the project owner gets the CERs issued by the EB.

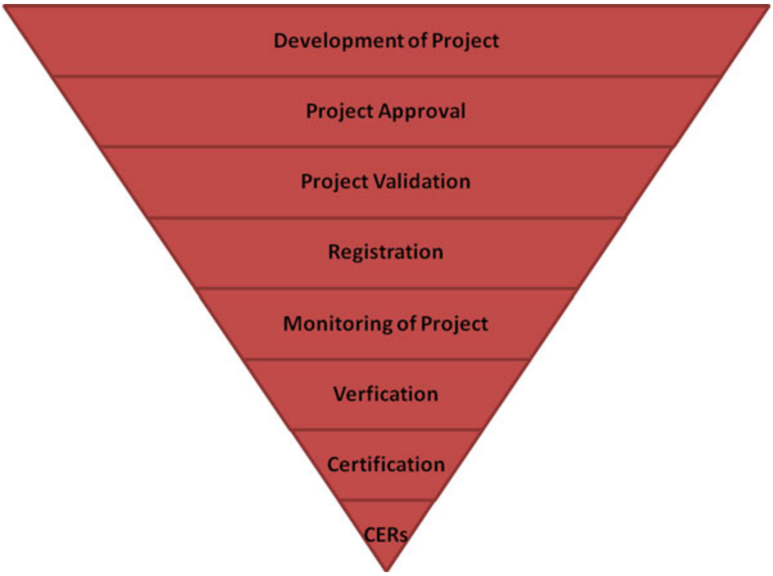

Fig. 3 Project cycle of CDM (Nautiyal and Varun 2012)

If a project developer got one CER, i.e. 1 tonne of emissions reduction in the CDM project, then he or she can produce 1 tonne of emission in another project (business-as-usual project) having no contribution in emissions reduction.

CDM projects may be classified into large-scale CDM projects, small-scale CDM projects, forestry projects and programme of activities or programmatic CDM projects (UNEP 2010). Renewable energy projects are found more suitable for achieving the main objectives of CDM. The use of renewable energy sources is more beneficial due to their property of nondepletion and low emissions generation. Renewable energy generation is also important for accomplishing the high energy demand of the world's population. This is due to the fact that dependency on conventional energy sources or petroleum products (coal, natural gas, etc.) for generating power is not expedient because their fast depletion nature, high prices and high emissions are the three main barriers in generating power without any environmental problem. So the use of conventional energy sources is not appropriate for sustainable development. Therefore, there is a great need to think and promote some other alternatives of energy so that the energy demand of the world's population can be achieved in a sustainable manner. Renewable energy sources are a viable option to achieve sustainable energy development (Lund 2007). They are amply found in many developing countries in the world (Ehnberg and Bollen 2005). As a consequence of this, all nations of the world are trying to explore and promote renewable energy sources for clean energy generation (Robinson 2004). Solar energy, hydropower, biomass and wind are the popular renewable energy sources. In addition to this, the world is also focussing in harnessing energy from geothermal sources and oceans (tidal energy). Table 2 shows the status of renewable energy-based power generation, and the total renewable energy capacity of the world in 2012 is 1,470 GW.

The main objective of promoting renewable energy technologies is sustainable development so that environmental problems like climate change and high emissions can be controlled. Also, CDM was introduced to reduce the same environmental problems and achieve sustainable development. There is a good link between renewable energy and CDM, and both are beneficial to achieve sustainable development altogether (Wohlgemuth and Missfeldt 2000). Therefore, promotion of renewable energy projects is very essential to promote CDM and

Table 2 Renewable energy power generation, 2012 (units in GW) (Renewable Global Status Report 2013)

Source	EU[a]-27	China	The United States	Germany	Spain	Italy	India	World's total
Wind power	106	75	60	31	23	8.1	18.4	283
Hydropower	119	229	78	4.4	17	18	43	990
Solar power (PV)	69	7	7.2	32	5.1	16.4	1.2	100
Biopower	31	8	15	7.6	1	3.8	4	83
Geothermal power	0.9	–	3.4	–	–	0.9	–	11.7
Tidal power	0.2	–	–	–	–	–	–	0.5
Concentrating solar thermal power (CSP)	2	–	0.5	–	2	–	–	2.5
Total	330	319	164	76	48	47	67	1,470

[a]European Union

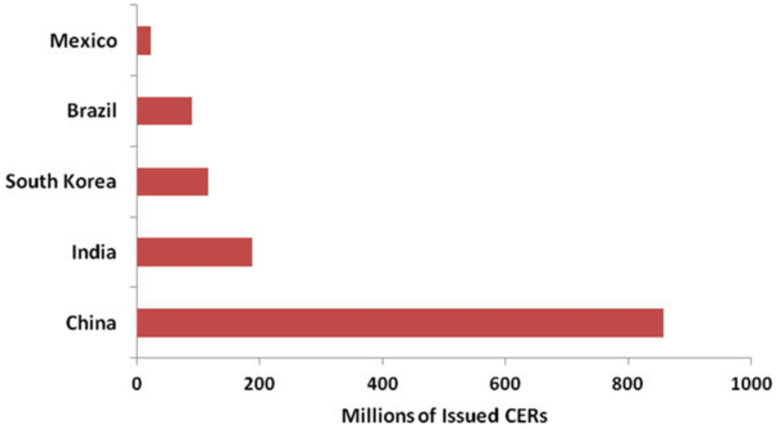

Fig. 4 Top five countries on the basis of issued CERs

emission trading so that emissions can be reduced considerably.

All the developed countries are now trying to get CERs to follow the Kyoto Protocol by promoting CDM projects. With the help of high amounts of CERs, countries can run their business-as-usual projects. Consequently, there is a noticeable growth in the establishment of CDM projects; with this the trouble of high GHG emissions can be reduced. Figure 4 shows top five countries on the basis of issued CERs. China has the highest CERs, leading with 857.4 million CERs, followed by India, South Korea, Brazil and Mexico. The increase in the number of CDM projects registered throughout the world year by year is a good sign in the path to sustainable development (www.cdmpipeline.org).

3 Barriers in CDM

Before implementing a CDM project, an estimation of emissions reduction is done which is then verified by a DOE. Monitoring of emissions is the fifth stage of the CDM project cycle as shown in Fig. 3 in which a project developer estimates the total emissions reduction of a project. After this stage, a DOE verifies the emissions reduction claimed by the project developer. But an important thing arises here: on what basis or standard does a DOE verify the claimed emissions reduction of the developer? That is why some standard or basis is essentially required for better verification of emissions reduction.

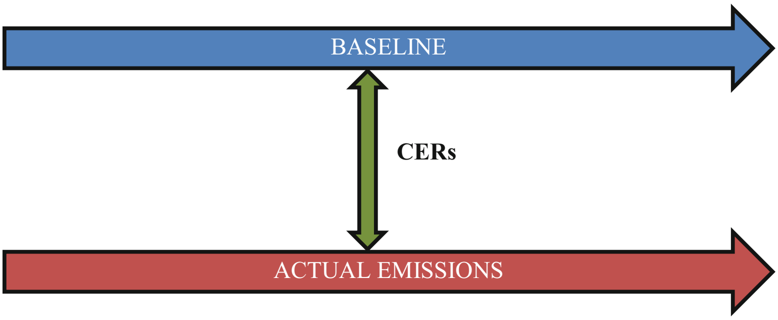

Fig. 5 CER estimation by baseline

Baseline methodology is commonly used to verify the monitoring report of a CDM project. First, it is essential to discuss the "baseline" which is defined as the amount of GHG emissions that would be produced if the CDM project is not there. The difference between baseline emissions and the actual emissions associated with a CDM project gives CERs. The CER calculation using baseline methodology is shown in Fig. 5. It can be noticed from the figure that the estimation of CERs using baseline methodology is quite simple and easy, but in actual practice, it is not so. This is due to the difficulty in the proper and correct establishment of a baseline. Actually, the concept of baseline is hypothetical in nature because the total emissions reduction, assuming the absence of a CDM project, is only an approximate estimation. Therefore, several baselines are possible depending upon the factors taken in the estimation of emissions reduction without the implementation of CDM projects. However, there are some common EB-approved methodologies for the development of a baseline. The three main approaches in the development of a baseline are using some past emissions data, emissions associated with a technology that represents an economic course of action taking investment barriers and the last 5 years of average emissions data related to the same project activities in the same social, economic, environmental and technological conditions and whose performance is among the top 20 % of their category (UNEP 2010).

The second important thing to be noted in a CDM project which has been discussed earlier is if a project developer has got one CER, i.e. 1 tonne emissions reduction in a CDM project in a host country, then he or she can produce 1 tonne of emission in his or her other project. It means that at one place, 1 tonne of emission is reduced but at some other place 1 tonne is still produced. So what is the net reduction in emissions in the climate? More or less the answer is "nothing". In other words, it can be said that at one place some sustainable output (having low emissions associated with a CDM project) and in another place emissions are still being produced into the atmosphere. This shows that the development of CDM projects means the development of projects based on some nonconventional sources of energy. In this regard, the overall outcome is the development of sustainable energy projects with the development of CDM projects. The conclusion is that a project developer is interested in the promotion and development of CDM projects because he or she can still run his or her business-as-usual projects with high emissions generation. And he or she is getting CERs effectively for business-as-usual projects. Due to this problem, the concept additionality arises in CDM, and it becomes a primary condition for the validation of a CDM project. Additionality must be considered during a CDM project analysis to check if the project is additional or not.

The next barrier in the development of CDM-based projects is that rural and remote areas are still far away from CDM. Project developers are not so much interested to developing CDM-based projects in rural areas. The main reason behind this issue is that rural areas are not well developed. In addition to this, many financial barriers arise from the establishment of renewable energy projects in rural areas. Arrangement of energy sources, proper infrastructure and transportation are the main challenges associated with the development of CDM projects in rural areas. Due to these problems, the establishment of CDM projects in rural areas becomes highly expensive. Consequently, progress in CDM-based projects in rural areas is not appreciable.

4 Conclusion

Climate change is a primary environmental problem which occurs due to the generation of large amounts of GHG emissions into the atmosphere. Both developed countries (Annex I countries) and developing countries (non-Annex I countries) must take a serious action to control GHG emissions and promote a sustainable and eco-friendly environment. An agreement was implemented in 1997, known as the Kyoto Protocol, which set binding rules for Annex I countries to control emissions. CDM is an important mechanism given by the Kyoto Protocol which allows emission trading and helps Annex I countries to control emissions and promote sustainable energy development in non-Annex I countries. Renewable energy-based projects are a good alternative to achieve the objectives of CDM. They are becoming more popular and viable throughout the world due to their nondepleting and environment-friendly nature. However, there are some barriers associated with the promotion of CDM projects. The estimation of the reduction in GHG emissions from CDM projects has some difficulties. EB has given some guidelines to reduce the difficulties associated with the determination of the actual baseline and additionality, and research is still going on to reduce the impediments related to the enhancement of CDM projects.

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Microalgae as an Attractive Source for Biofuel Production

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Abstract

With the depletion of fossil fuel resources and the limited availability of petroleum-derived transport fuel, along with the contribution to global warming, the environmental benefits of renewable biofuel are seen as the best alternative source in recent years. Among the third-generation biodiesel feed stocks such as food crops (sugarcane, sugar beet, maize and rapeseed) and non-food crops (*Jatropha* sp., *Cassava* sp., lignocellulosic materials), microalgae has been hailed as the third-generation biodiesel. Microalgae are the only fuel source that can be sustainably developed in the near future, and can produce ten times more oil than oleaginous plants. Biodiesel from microalgae has received much attention world-wide in recent years due to its carbon-neutral status. The higher neutral lipid contents of microalgae also surpass terrestrial plants for biofuel production, and microalgae are the largest biomass producers. They can accumulate high concentrations of triacylglycerol as a storage lipid under photooxidative stress and other unfavorable environmental conditions within a short

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period of time. This chapter provides an overview of the production of biodiesel from microalgae and includes algae cultivation, biomass production, harvesting, and downstream processing, along with a list of companies aiming to develop biodiesel from microalgae.

Keywords
Microalgae • Biomass • Biofuel • Transesterification • Triacylglycerol

1 Introduction

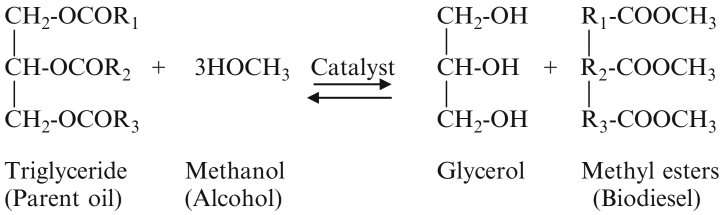
Fossil fuels are the largest contributor of greenhouse gases. They are about to reach peak production and are predicted to be exhausted in the future due to their limited and non-renewable nature. Therefore, the current use of fossil fuels is widely recognized as unsustainable (Hemaiswarya et al. 2012). In the year 2007, the US Department of Energy reported that the burning of fossil fuels produces around 21.3 billion tonnes (≡gigatonnes [Gt]) of carbon dioxide (CO_2) per year; however, it is estimated that natural processes can only absorb about half of that amount, resulting in a net increase of 10.65 billion tonnes of atmospheric CO_2 per year. Carbon dioxide is one of the greenhouse gases that enhances radioactivity and contributes to global warming (a rise in the average surface temperature of the earth), which a majority of climate scientists agree is going to cause major adverse effects. Data show that, between 1989 and 2008, the proven oil reserves seem to have increased from 1,006 to 1,333 billion barrels. The annual world primary energy consumption was measured at 12,274.6 million tonnes of oil equivalent (MTOE). Fossil fuels accounted for 87.08 % of primary energy consumption, with the main fuels being oil (33.07 % share), coal (30.34 %), and natural gas (23.67 %); the minor fuels account for 17.08 % of primary energy consumption, and include nuclear energy (4.15 %), hydroelectricity (6.45 ,%), and renewables (6.48 %) (BP 2011). On the other hand, while the world oil consumption was about 86 million barrels/day in 2006, it is estimated to reach 107 million barrels/day by 2030.

Dependency on fossil fuel, primarily in the transportation sector, has encouraged research on biofuels. One-fifth of global CO_2 emissions is from the transport sector. The global number of vehicles has been roughly estimated to rise to two billion by the year 2050 (Balat and Balat 2010). The transportation sector would be responsible for 80 % of this increase (US Energy Information Administration 2009) and would consume 76 % of the world oil production by 2030 (International Energy Agency 2008). A recent study has shown that microalgae biofuels have the potential to replace 17 % of oil imports used for transportation fuel in the USA by 2022 (Wigmosta et al. 2011). Moreover, following the BP oil spill in the Atlantic Ocean, the US administration is considering reducing its oil imports by one-third by 2021, using, among others, biofuels to do so (Banerjee 2011). The only possible alternative to overcome all these problems is to produce ‘algal biodiesel’.

2 Biodiesel

Biodiesel is a monoalkyl ester; the basic chemical reaction required to produce biodiesel is the transesterification of lipids, either triglycerides or oil, with alcohol (Fukuda et al. 2001). The result is a fatty acid alkyl-ester, which is the biodiesel material used in engines (e.g. fatty-acid methyl-ester [FAME]); this reaction is performed at high pH. The alcohols used include methanol and, to a lesser extent, ethanol; the main by-product is glycerol. This chemical reaction is sensitive to water. The quality and productivity of biodiesel is affected by the presence of water, because saponification reactions occur (soap formation) when water combines with the lipid. Excess unsaturated fatty acid levels are a major problem in biodiesel production because they may induce cross-linking of fatty acid chains, causing the formation of tar.

Poly-unsaturated fatty acids (PUFAs) are a potential co-product of biodiesel from microalgae. PUFAs from microalgae are a vegetable origin alternative to fish oils and other oils rich in omega-3 fatty acids. In the biodiesel process, PUFAs would be extracted prior to esterification, as these fatty acids are not the most suitable raw material for esterification. As far as biodiesel esterification is concerned, the main by-product is glycerol. Glycerol is a versatile chemical, with over 1,500 known commercial applications, though this market has become somewhat saturated due to strong growth in worldwide biodiesel production. Glycerol could be used for mixed fermentation together with sugar and protein residues from the lipid extraction step.



2.1 Advantages of Biodiesel

For several reasons, biodiesel fuel seems to be an alternative energy resource. The advantages of using biodiesel fuels are as follows.

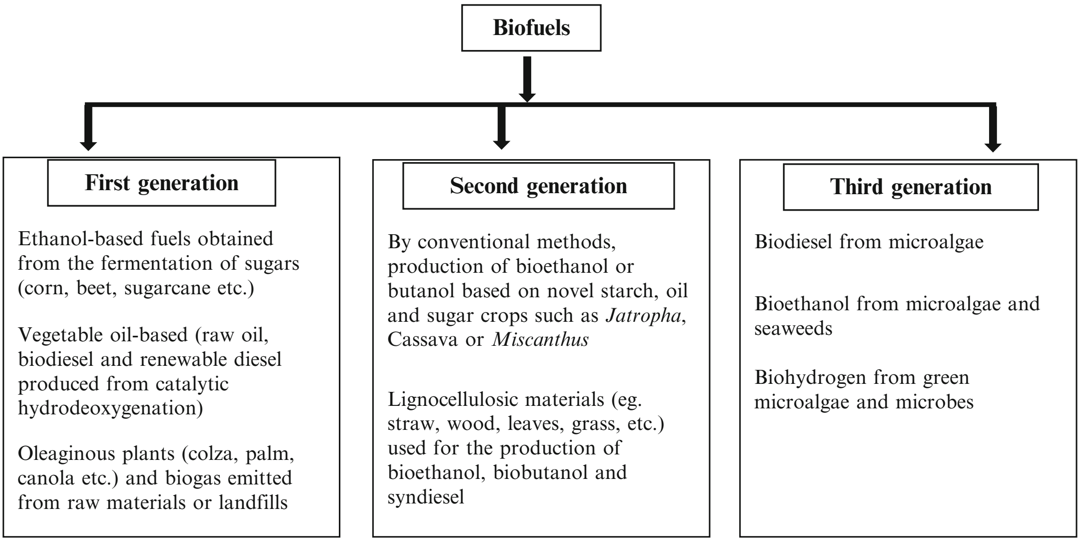
Biodiesel has higher lubricity than petroleum diesel and is a renewable energy resource that could be sustainably supplied. It is understood that the petroleum reserves are to be depleted in less than 50 years at the present rate of consumption (Sheehan et al. 1998).

Biodiesel appears to have several favorable environmental properties: a low release of CO_2 and very low sulfur content (Antolin et al. 2002; Vicente et al. 2004). The release of sulfur and carbon monoxide would be reduced by 30 and 10 %, respectively, with the use of biodiesel. Additionally, the use of biodiesel as an energy source can reduce the level of gas that is generated during combustion, and the relatively high oxygen content of biodiesel can decrease the level of carbon monoxide. Moreover, biodiesel does not contain aromatic compounds and other chemical substances that are harmful to the environment. Recent research (Sharp 1996) has indicated that 90 % of atmospheric pollution and 95 % of cancers can be decreased with the use of biodiesel.

Biodiesel appears to have significant economic potential; the price of fossil fuels will undoubtedly increase in the future because of their non-renewable nature (Cadenas and Cabezudo 1998). Finally, the flash point and biodegradability of biodiesel is better than diesel fuel (Ma and Hanna 1999).

2.2 Sources of Biodiesel Production

The current sources of commercial biodiesel include soybean oil, palm oil, animal fat, and waste cooking oil. These sources can be classified into three generations.



Although first-generation biofuel production capacities have increased, they have considerable economic and environmental limitations. A lack of agricultural land and deforestation (National Research Council 2007; Goldemberg and Guardabassi 2009) has led to competition with agriculture for arable land used for food production. For example, in some European countries such as France, production of first-generation biofuels using arable land available from the cultivation of oleaginous plants will not be able to support demand for biofuels by 2015 unless fallow land is saturated, which in turn would create soil impoverishment problems. According to the International Energy Agency (IEA 2008), about 1 % (14 million hectares) of the world's available arable land is used for the production of biofuels, providing 1 % of global transport fuels. This clearly shows that an increase in the share to anywhere near 100 % is unfeasible due to the severe impact on the world's food supply and the large areas of production land required.

Second-generation biofuels contain high amounts of free fatty acids; however, additional production steps demand increased energy and production costs (Demirbas 2008). The major limitation associated with the use of second-generation biofuels is the issue of sustainability. For biodiesel to be produced from *Jatropha*, half of the land area of the UK (17.5 million hectares) would be required to fulfill the diesel requirement of the UK (estimated 25 billion liters in 2008). The total energy input for the life cycle of rapeseed and soybean is estimated to be half the total energy of the fuel (Scott et al. 2010). The third largest biodiesel feedstock is palm oil. Universally, 10 % of palm oil is being used as a biodiesel source. The major disadvantage of palm oil biodiesel is poor cold flow properties, which can be overcome by blending with triglycerides to improve cold flow and cloud point properties (Sarin et al. 2009). High production of palm oil requires vast areas of natural vegetation; it can also indirectly cause damage to the ecosystem due to devastating fires. In addition, it has a negative impact on terrestrial and aquatic environments due to palm mill effluent (Ahmad et al. 2011).

Production of first- and second-generation biodiesel from crops is not affordable. Therefore, competition between biodiesel feedstock production and food production mean it is necessary to find other ways to lower the cost of biodiesel production and reduce the pressure on food and feed supplies. Biodiesel also has one major limitation: it does not fully replace other petroleum diesel and biomass-based diesels, meaning that one gallon of biodiesel does not have the same physical properties (temperature stability, energy content, etc.) as petroleum diesel and other biomass-based diesels (National Renewable Energy Laboratory 2009). Therefore, biodiesel can only be used interchangeably to a limited extent in the form of blends and in the few engines that are specifically designed to handle pure biodiesel (B100).

The reason for the differences in physical characteristics is the different types of molecules that constitute biodiesel and petroleum diesel. Biodiesel is composed of straight chain hydrocarbon esters that have one oxygen atom per molecule; petroleum diesel is composed of non-oxygenated straight and cyclic hydrocarbon chains. Biodiesel does not meet all of the stability, distribution, and engine requirements of standard diesel fuels because the compound includes oxygen, called esters. The use of pure biodiesel in the existing infrastructure can cause problems during transportation through pipelines and when used in engines; however, these difficulties can be managed without a substantial amount of monetary costs (McElroy 2007). A comparison between petro-diesel and biodiesel is shown in Table 1.

Table 1 Comparison between petrodiesel and biodiesel

Properties	Petrodiesel	Biodiesel
Lower heating value (Btu/gal)	~129,050	~118,170
Kinematic viscosity @ 40 °C (mm^2/s)	1.3–4.1	1.9–6.0
Specific gravity @ 40 °C (Kg/l)	0.85	0.88
Density (lb/gal)	7.079	7.328
Water and sediment (% volume)	0.05 max	0.05 max
Carbon (wt.%)	87	77
Hydrogen (wt.%)	13	12
Oxygen	0	11
Sulfur (wt.%)	0.0015	0.0–0.0024
Boiling point (°C)	180–340	315–350
Flash point (°C)	60–80	130–170
Cloud point (°C)	−15 to 5	−3 to 12
Pour point (°C)	−35 to −15	−15 to 10
Cetane number	40–55	47–65
Oxidative stability	–	3–6 min
Lubricity SLBOCLE (grams)	2,000–5,000	>7,000
Lubricity HFRR (microns)	300–600	<300

Adapted from Crimson Renewable Energy, LP; http://www.crimsonrenewable.com/biodiesel-specifications.pdf; Knothe (2010) and Hemaiswarya et al. (2012)
HFRR = High Frequency Reciprocating Rig, SLBOCLE = Scuffing Load Ball-on-Cylinder Lubricity Evaluator

Microalgae are one of the most promising alternative and renewable feedstock sources for production of third-generation biodiesel. Microalgae have been found to have incredible oil production levels when compared with other oil seed crops such as soybeans, palm oil, etc. Biodiesel from microalgae is a sustainable development as they are carbon neutral, or reduce atmospheric CO_2 as they are carbon negative (Naik et al. 2010). It is estimated that 1.8 tonnes of CO_2 would be consumed (180 % reduction) by each tonne of microalgal biomass produced. This chapter mainly focused on microalgae as a potential source of biodiesel production.

3 Microalgae

Microalgae are microscopic aquatic (freshwater or marine forms) photosynthetic plants that require the aid of a microscope to be seen and can be measured in microns. Microalgae have the right oils to be converted to biodiesel. However, a microscope becomes superfluous when it comes to visualizing the potential of these organisms for biofuel production (Fig. 1a–e). With improved oil and biomass yield, algae can produce a considerably higher level of biomass and lipids per hectare than terrestrial biomass. Most microalgae are found in freshwater and marine environments; a few grow in terrestrial habitats. But the choice of microalgae species for cultivation is based on their lipid and biomass productivity as well as cultivation location. Marine and freshwater species have shown similar biomass and lipid productivities, thus making strain selection dependent on other factors (Ahmad et al. 2011). Some of the fresh and marine water microalgae and their lipid productivity (% dry weight) are shown in Table 2.

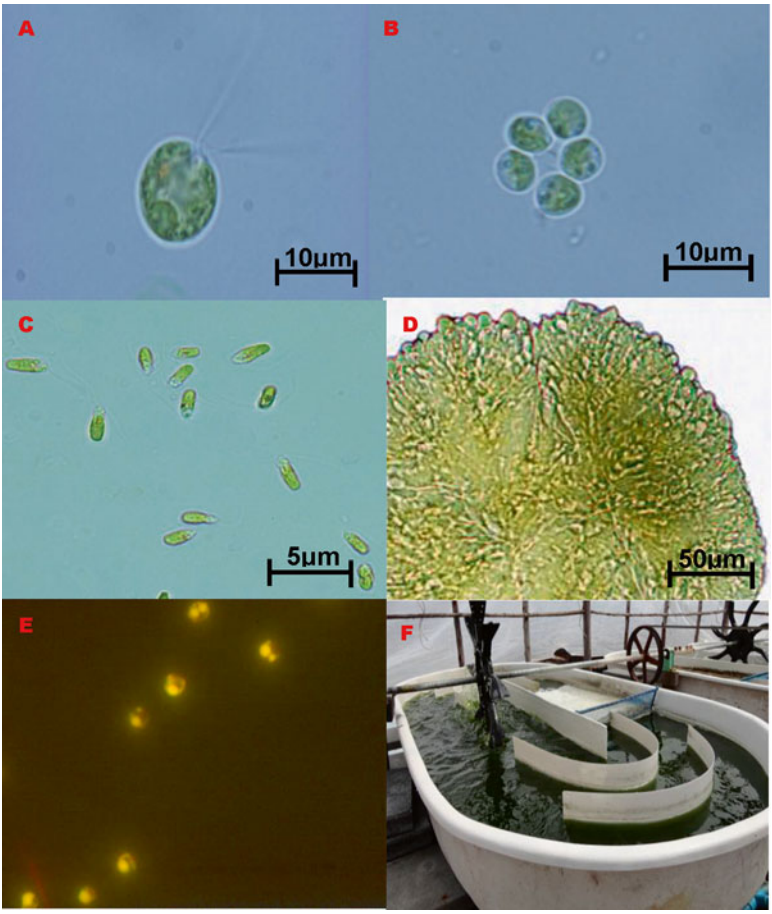


Fig. 1 Morphological structures of some microalgae. (**a**) *Chlamydomonas* sp., (**b**) *Chlorella* sp., (**c**) *Dunaliella* sp., (**d**) *Botryococcus braunii*, (**e**) *Chlorella vulgaris* stained by Nile red, (**f**) culturing microalgae in mini raceway ponds

3.1 Freshwater Algae

Freshwater algae are found growing underwater on rocks, in ponds and lakes, and in mud in streams and rivers. They are usually more abundant in slower than in fast flowing streams. In inland areas, cultivation of fresh water microalgae may be more suitable and will not affect the fertile (agricultural) area in the same way as marine algae. The major drawback is contamination, and cultivation of freshwater species allows a more diverse number of species to be transmitted. There are many types of freshwater algae, identified as green algae (Chlorophyta), red algae (Rhodophyta), and diatoms (Bacillariophyta). Green algae look like strands of green hair flowing in the current. Diatoms can appear to the naked eye as brown mats of growth with soft masses or slimy layers on rocks.

3.2 Marine Water Algae

Marine forms are grown near coastal areas, salt marshes, brackish water, or even floating in the ocean. One successful way to improve the economics of biomass productivity of microalgae will be to cultivate marine microalgae (Uduman et al. 2011). Marine microalgae can be grown in saline waters, so a selective environment will serve to reduce

Table 2 Lipid contents and productivity of different microalgae species

Strains	M/F	Lipid content (% dry weight)
Botryococcus braunii	F	25–75
Chaetoceros calcitrans	M	14.6–16.4
Chaetoceros muelleri	M	33.6
Characium californicum	F	16.1
Characium oviforme	F	13.3
Chlamydomonas rheinhardii	F	21
Chlorella emersonii	F	25–63
Chlorella luteo-viridis	F	12.2
Chlorella protothecoides	F	14.6–57.8
Chlorella sorokiniana	F	19–22
Chlorella vulgaris	F	14–22
Chloridella neglecta	F	23
Chloridella simplex	F	31.8
Chlorococcum costazygoticum	F	20
Chlorococcum infusionum	F	21.4
Coccomyxa chodati	F	21.9
Coelastrella striolata	F	15.2
Crypthecodinium cohnii	M	20.0–51.1
Cylindrotheca sp.	M	16–37
Ellipsoidion sp.	M	27.4
Euglena gracillis	F	14–20
Haematococcus pluvialis	F	25.0
Isochrysis galbana	M	7–40
Lobochlamys culleus	F	16.5
Lobochlamys segnis	F	20.0
Monallanthus salina	M	20–22
Monodus subterraneus	F	16
Monoraphidium arcuatum	F	23.4
Monoraphidium contortum	F	22.2
Monoraphidium dybowskii	F	19
Monoraphidium griffithii	F	22.2
Monoraphidium neglectum	F	17.8
Monoraphidium terrestre	F	15.9
Monoraphidium tortile	F	31.5
Muriella aurantiaca	F	20.5
Nannochloris eucaryotum	M	18.4
Nannochloris sp.	M	21.4
Nannochloropsis oculata	M	22.7–29.7
Navicula salinicola	F	24.7
Neochloris oleoabundans	F	29–65
Nitzschia sp.	M	16–47
Parachlorella kessleri	F	22.8
Pavlova lutheri	M	35.5
Pavlova salina	M	30.9
Phaeodactylum tricornutum	M	18–57
Pleurastrum insigne	F	23.4
Porphyridium cruentum	M	9–14
Prymnesium parvum	M	22–38
Scenedesmus costatus	F	11.9
Scenedesmus dimorphus	F	16–40
Scenedesmus obliquus	F	12–14
Scenedesmus quadricauda	F	1.9–18.4
Schizochytrium sp.	M	15–23
Skeletonema costatum	M	13.5–51.3
Synechoccus sp.	M	11
Tetraselmis suecica	M	8.5–23.0
Thalassiosira pseudonana	M	20.6

Source adapted from Rodolfi et al. (2009), Mata et al. (2010), Mutanda et al. (2011), and Bogen et al. (2013)
Note: M/F indicates marine or freshwater species

extensive contamination. Another important factor for consideration is water availability (Mata et al. 2010). By growing marine microalgae, sea water can be used directly as an alternative to fresh water (Amaro et al. 2011). There are some disadvantages to using sea water for the growth of microalgae; sea water generally consists of marine flora that may consume microalgae. For the growth of microalgae, a large amount of water is needed to remove micro flora, or the filtering of sea water will negatively impact on the economics of production. Higher evaporation rates could result in an increase in salinity, thus the necessary adjustment of culture condition with freshwater incurs an additional cost. Osmotic shock and rupturing of cells under higher salinity may not be suitable for lipid recovery (Mata et al. 2010).

4 Why Microalgae Are Attractive

Microalgae are easy to cultivate in an aquatic medium and need less water than terrestrial crops. They can be cultivated in seawater or brackish water on non-arable land. Microalgae have a high growth rate, a short life cycle, and can be harvested continuously (Chisti 2007; Schenk et al. 2008). Microalgae double their biomass within 24 h. In fact, the biomass doubling time for microalgae during exponential growth can be as short as 3.5 h (Chisti 2007), which is significantly quicker than the doubling time of oil crops. They have the ability to reproduce themselves using simple photosynthesis to convert solar energy into chemical energy and accumulate their lipids in the form of oil, carbohydrate, and protein etc. (Schenk et al. 2008). Microalgae can grow in simple nutrients and sunlight, although the growth rate will vary with the addition of specific nutrients and aeration (Renaud et al. 1999; Pratoomyot et al. 2005; Aslan and Kapdan 2006). Many microalgae accumulate high levels of oil, which can be converted into biodiesel. Microalgae can accumulate more than 80 % of lipids on the dry weight of biomass (Chisti 2007). According to the US Department of Energy, the oil yield of algae is 10–100 times higher than conventional oilseed crops (soy, rapeseed, or tree-borne oil plantations such as *Jatropha* and palm). Microalgae have a high theoretical production yield of 47,000–3,08,000 L ha^{-1} $annum^{-1}$; whereas the oil palm has the ability to produce 5,950 L of biodiesel per year (Ahmad et al. 2011).

Microalgae biomass, either terrestrial or aquatic, is considered one of the best alternative renewable energy sources (Chisti 2008; Raja et al. 2008). Microalgae biomass production may be combined with direct bio-fixation of waste CO_2 (1 kg of dry algal biomass requiring about 1.8 kg of CO_2). Depending on the species of microalgae, other compounds that may also be extracted include a wide range of fine chemicals and bulk products such as fats, PUFAs, oil, natural dyes, sugars, pigments, antioxidants, high-value bioactive compounds, and algal biomass, which are valuable applications in the industrial sector (Li et al. 2008a, b; Raja et al. 2008; Sathasivam et al. 2012). These high-value biological derivatives with many possible commercial applications mean that microalgae can potentially be used in a large number of biotechnology areas, including biofuels, cosmetics, pharmaceuticals, nutrition and food additives, aquaculture, and pollution prevention (Raven and Gregersen 2007; Rosenberg et al. 2008; Jacob-Lopes and Teixeira Franco 2010).

5 Classification of Microalgae Based on Metabolism

Microalgae can be divided into four major groups of algal metabolism: photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic (Chen et al. 2011). A short explanation follows.

5.1 Photoautotrophic Microalgae

Photoautotrophic microalgae build up CO_2 and water into organic cell materials using energy from sunlight. In this condition, microalgae absorbs energy from light and stores it in the form of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) to produce glucose in a Calvin cycle. However, inadequate light and CO_2 supply are always an issue for photoautotrophic cultures. It has been reported that the addition of excess CO_2 into the culture system may increase the microalgae lipid and biomass productivity, but care should be taken because an excess amount of CO_2 is released in the atmosphere. Light also plays an important role because the uneven distribution of light intensity will directly affect the growth of microalgae. For example, biomass productivity is lower in photoautotrophic species than heterotrophic microalgae in both photobioreactor and open ponds—between 0.117 and 1.54 $kg/m^3/day$, respectively (Chisti 2007).

5.2 Heterotrophic Microalgae

Heterotrophic microalgae require at least one organic nutrient from organisms or their by-products, as a carbon source for producing their own organic compounds. Heterotrophic cultures utilize organic wastes containing carbons as an energy source instead of CO_2 and are independent of the light source for reproduction. Thus, the high production of lipids is enabled compared with phototrophic microalgal cultivation. Heterotrophic microalgae have the ability to accumulate a higher level of lipids than photoautotrophic microalgae (Xiong et al. 2008; Liang et al. 2009). Although it accumulates higher lipids, it is not economically feasible when the carbon sources have to be purchased, as this directly increases production costs. Carbon sources used for culturing algae include glycerol, glucose and sweet sorghum, corn powder hydrolyzate, and Jerusalem artichokes. This glucose has been widely used as a carbon source for microalgal cultivation. The most suitable glucose concentration for a high accumulation of lipids is 2 %. For example, with a glucose concentration of 0.5–8 %, the carbon source showed 44.48 % lipid content (Wu et al. 2005), whereas the glucose concentration in the normal medium does not exceed 2.4 % and accumulates lipid content as high as 57 % (Xiong et al. 2008). Sweet sorghum is another carbon source used for culturing algae. The addition of 50 % sweet sorghum juice into the culture medium instead of glucose achieved a lipid content as high as 73.4 % (Liang et al. 2010). Heterotrophic species are one of the best for the production of biodiesel (Xu et al. 2006; Martek 2008; Xiong et al. 2008). For example, *Chlorella* sp. grown in heterotrophic conditions showed a high level of biomass (7.4 kg/m^3/day) and lipid content 50–58 % (g lipid/g dry weight) (Xiong et al. 2008).

5.3 Photoheterotrophic Microalgae

Photoheterotrophs are also referred to as photoorganitrophs, photoassimilates, and photometabolism. Photoheterotrophic microalgae use light, but obtain their carbon in organic form. For example, microalgae under stress conditions produce glycerol; these glycerols are used as a carbon source and it utilizes a light intensity of 35 μE/m^{-2}/s^{-1}. Yang et al. (2011) recorded an increase in the biomass concentration of *Chlorella minutissima* (UTEX2341) from 1.2 to 8.2 g/L.

5.4 Mixotrophic Microalgae

Mixotrophic microalgae combine both an autotrophic and a heterotrophic metabolism, simultaneously assimilating CO_2. *Ch. vulgaris* grown with both light and glucose sources showed higher biomass productivity (254 mg/L/day). When grown in the dark under CO_2 instead of glucose, the biomass productivity (151 mg/L/day) was low (Liang et al. 2009; Chen et al. 2011). Among all the mixotrophic culture conditions, algae accumulate high lipid contents even though they are rarely used in microalgal oil production. According to Cheirsilp and Torpee (2012), when freshwater *Chlorella* sp., marine *Chlorella* sp., *Nannochloropsis* sp., and *Cheatoceros* sp. were grown in mixotrophic cultures, a much higher biomass and lipid level was produced than the photoautotrophic and heterotrophic cultures.

The presence of different metabolisms can be distinguished according to pH flexibilities, which depends on the growth stoichiometry of microalgae as part of the metabolism involved. In the autotrophic metabolism, due to the consumption of CO_2, the growth medium becomes alkalized, whereas, in heterotrophy, where CO_2 is produced from an organic carbon source, the growth medium becomes acidulated. In a mixotrophic culture, the pH value depends on the dominating constituent metabolism, but in most cases remains constant. Some strains also have the ability to grow under photoautotrophic, heterotrophic and mixotrophic conditions (e.g. *Ch. vulgaris*, *Haematococcus pluvialis*, *Arthrospira* (*Spirulina*) *platensis*). Other strains, such as *Selenastrum capricornutum* and *Scenedesmus acutus*, can grow either photoautotrophically, heterotrophically, or photoheterotrophically (Chojnacka and Marquez-Rocha 2004).

6 Screening of Lipid Production Under Different Environmental Conditions

Screening of microalgae producing high levels of oil is one of the most important criteria in the optimization of biodiesel production. Oil content in microalgae can be stored as high lipid contents up to a maximum of 75 % (g lipids/g dry weight) biomass, but this is associated with low productivity. Some cultures accumulate a moderate amount of lipids (20–50 %) and, when conditions are optimized, higher productivity can be reached. The lipid content (% dry weight) of different marine and freshwater microalgae species has shown significant differences among the species (Table 2). The other factors are taken into account, such as lipid profile and the ability to grow under specific environmental conditions. The factors that affect the lipid profile are nutritional, processing, and cultivation conditions. Factors such as growth rate, quality and quantity of lipid, and strong adaptability in a changing environment to determine the preferred nutrients and nutrient uptake rates are very important in selecting better strains (Amaro et al. 2011).

The composition of fatty acids plays an important role in the characteristics of the biodiesel they produce and differs between species. Biodiesel is composed of saturated and unsaturated fatty acids with 12–22 carbon atoms, some of them of ω3 and ω6 families. The seven freshwater microalgae showed similar fatty acid composition patterns, showing that all microalgae synthesize C14:0, C16:0, C18:1, C18:2, and C18:3 fatty acids. The relative intensity of other individual fatty acid chains is species specific, e.g. *Ankistrodesmus* sp. consists of C16:4 and C18:4; *Isochrysis* sp. consists of C18:4; and *Nannochloris* sp. consists of C22:6, C16:2, C16:3, C20:5, C16:2, and C16:3; and *Nitzschia* sp. consists of C20:5 (Thomas et al. 1984; Mata et al. 2010).

Some microalgae oil yield is higher than vegetable oil; this is strain dependent. Different nutritional and environmental factors may affect the fatty acid composition. The high amount of oil production is induced under stress conditions such as nitrogen deficiency, high light intensity, phosphorous deficiency, silicon deficiency, salt stress, and other factors. For example, *Botryococcus braunii* under nitrogen deficiency and salt stress induced the accumulation of C20:5 whereas, all other species accumulated C18:1 (Őtleş and Pire 2001; Pratoomyot et al. 2005; Natrah et al. 2007; Hu et al. 2008; Gouveia and Oliveira 2009; Mata et al. 2010). Some microalgae have the ability to accumulate lipids under nitrogen deprivation. *Nannochloropsis* sp. attained 204 mg/L/day (with an average biomass productivity of 0.30 g/L/day and more than 60 % lipid content) in nitrogen-deprived media (Rodolfi et al. 2009; Veillette et al. 2012). *Navicula* accumulates 58 % (g lipid/g dry weight) under nitrogen deficiency, whereas the lipid content is very low (i.e.) 22–49 % (g lipid/g dry weight) under silicon (Si) deficiency. The above results clearly prove that nitrogen deprivation has a positive effect on lipid accumulation.

On the other hand, phosphate deprivation could have an effect on lipid content (Khozin-Goldberg and Cohen 2006; Xin et al. 2010). For example, when the P concentration was increased from 0.14 to 0.37 mg/L, an increase in the biomass was recorded, while the lipid content decreased from 53 to 23.5 % (g lipid/g dry weight). Silicon depletion also leads to an increase in the cellular lipid content. Under silicon starvation in the diatom, an increase of 60 % of the lipid content of *Navioua pelliculosa* is possible. Lipid fractions as high as 70–85 % on a dry weight basis have been reported. Such high lipid contents exceed that of most terrestrial plants. How it is that diatoms rapidly switch over from carbohydrate accumulation to lipid accumulation remains unclear. However, diatoms have great potential to accumulate microalgal lipids (Miyamoto 1997). At the initial stage of growth, more polar lipids and polyunsaturated C_{16} and C_{18} fatty acids are produced. The stationary growth phase generally consists of neutral saturated 18:1 and 16:0 long-chain fatty acids.

Light also plays an important role in lipid accumulation. Under high light intensity, *Euglena gracilis* and *Ch. vulgaris* produce polyunsaturated C_{16} and C_{18} fatty acids as well as mono- and di-galactosyl-diglycerides, sphingolipids, and phosphoglycerides. Under low temperature conditions, *Monochrysis lutheri* produce polyunsaturated C_{18} fatty acids, but in *Dunaliella salina* the fatty acid composition changes (Takagi et al. 2006). The above findings clearly deny that light intensity also plays a crucial role in fatty acid composition. Osmotic shock might also enhance lipid production. However, salt stress may affect the quantity of lipid within the algal cells. For example, *Dunaliella* cells can grow well in high

NaCl concentrations. Takagi et al. (2006) observed that *Dunaliella* cells grown in concentrations of >1.0 M NaCl show increased biomass, but there is no significant effect on biomass in a concentration of <1.0 M NaCl. However, intracellular lipid and triglycerides were higher (67 and 56 %) in saltwater species grown in 1.0 M NaCl; the same species grown in 0.5 M NaCl accumulates a low lipid content (60 and 40 %). However, these physico-chemical treatments could also favor the synthesis of polar lipids like phospholipids or glycolipids associated with cell walls of the microalgae; such lipids are less interesting for biodiesel production (Nagle and Lemke 1990).

7 Microalgal Lipid and Biomass Productions

The medium composition is an important factors for increased microalgal growth and lipid production. Microalgae require inorganic nutrients in the form of carbon, nitrogen, and phosphorous for growth (Suh and Lee 2003; Brennan and Owende 2010; Sathasivam and Juntawong 2013). The growth medium of microalgae generally consists of macronutrients like nitrogen, phosphate, and a metal chelator. Iron is generally a micronutrient, and low concentrations of iron in a form that can be assimilated are essential for microalgae growth. In a nutrient-rich medium, *Chlorella* sp. are known to grow fairly. The growth of various species of *Chlorella* in Watanabe media containing 1.25 g/L KNO_3 was demonstrated by Illman et al. (2000). Changes in culture conditions may lead to changes in metabolic pathways and result in the production of neutral lipids (Singh et al. 2011). Sometimes nutrient-rich media may cause nutrient toxicity, with lethal results (Watanabe et al. 2000). The well known microalgae and the medium used for their growth, biomass, and lipid productivity have been quoted from various literature. Stephenson et al. (2010) tested *Ch. emersonii* and *Ch. protothecoides* and achieved the highest lipid and biomass yield. Even though *Ch. vulgaris* and *Ch. minutissima* are capable of accumulating high lipid contents, lower triglycerides clearly indicated that the species is inefficient for use as a biodiesel feed stock.

Nannochloropsis has the ability to accumulate 60 % of lipids and if the culture is mixed with 2 % CO_2, the biomass yield is ~0.48 g/L/d (Vunjak-Novakovic et al. 2005; Chiu et al. 2009). These results indicate that the *Nannochloropsis* species have a potential use as biofuel feedstock, whereas the average lipid content and biomass yield of *Nannochloropsis* species is low when compared with *Schizochytrium limacinum*, *Ch. emersonii* and *Ch. protothecoides*. The lipid accumulation of *Chaetoceros calcitrans*, *Neochloris oleoabundans*, and *Scenedesmus obliquus* is very low (Natrah et al. 2007; da Silva et al. 2009). Even under nitrogen-deficit conditions, *N. oleoabundans* is able to accumulate 37 % of lipid/dry weight, and the biomass yield is in the range of 0.05–0.09 g/L/d (Pruvost et al. 2009). These results show that the algae are unproductive as biofuel feedstocks.

8 Global Scenario of Algal Biofuels

The growth of algae is motivated around the world (Piccolo 2009), and the large-scale algal biofuel companies throughout the world are listed in Table 3. Even though algae oil production has increased worldwide, the biggest algae investment in the EU is the £26 million in the public sector funded by the UK Carbon Trust. In 2009, the Carbon Trust launched a new £8 million research program called 'Algae Biofuels Challenge' (ABC) (http://www.carbontrust.co.uk/News/presscentre/2008/algae-biofuel-schallenge.htm). Later, the Scottish government launched a £6 million EU project called 'BioMara'. In 2007, a Spanish renewable energy company, Aurantia and Green Fuel Tech of Massachusetts (USA) formed a partnership through a $US92 million project to produce algae oil.

According to Piccolo (2009), countries with a coastline near the Mediterranean Sea region (roughly between 45°N and 30°N) are the best

Table 3 List of companies producing algae biomass and biofuel

Companies	Region	Technology (Process)	Feedstock	Primary product
A2BE Carbon Capture, LLC	USA	Algal photosynthesis/bio-harvesting	Algae	Biofuels, foods, and fertilizers
Albemarle Corporation	USA	Catalysis, polymer additives	Wood, municipal waste, agricultural residue, and algae	Ethanol; jet fuel, diesel, gasoline
Algae Aviation Fuel	USA	N/A	Algae	Bio jet fuel
Algae Bioscience	USA	Photobioreactor	Algae	Omega-3 fatty acid oils and high-protein algae meal
Algae Systems, LLC	USA	Algae cultivation systems, passive dewatering systems, downstream biorefining systems	Algae	Algal lipid
Algae.Tec	Australia	Bioreactor	Algae	Algal lipid
Algaeventure Systems	USA	Solid-liquid separation	Algae	Algal lipid
Algenol Biofuels	USA	Algae cultivated in the desert using sea water and CO_2. Fuel can be directly derived from the algal tanks while algae continue to thrive	Algae, blue-green algae, cyanobacteria	Fuel and oil
Aquaflow Bionomics	New Zealand	Cultivation and harvesting of algae from open air environment	Algae	Biofuel
Asiatic Centre for Genome Technology	Malaysia	Alga transesterification	Algae	Biodiesel
Aurora Algae	USA	Gasification	Algae	Protein, omega-3 oils, biodiesel, ethanol
Bay State Biofuels	USA	N/A	Algae	Biodiesel
Biocombustibles del Chubut	Argentina	N/A	Microalgae	Biodiesel
Biodiesel Industries	USA	N/A	Jatropha/ algae	Biofuels
Biofuel Advance Research and Development, LLC	USA	Closed loop system/photobioreactors	Jatropha/camelina/algae	Biodiesel, jet fuel
Biofuel system	Spain	Cultivation of algae by using natural setting cultivation method	Marine algae and phytoplankton	Biopetroleum
BioJet	USA	N/A	Jatropha, camelina, algae, waste biomass	Jetfuel
Biolight Harvesting	USA	N/A	Algae	Algal lipid
BioMara	UK	N/A	Marine biomass	Biofuel
BioMarine Fuels	USA	Bioreactor	Algae	Biodiesel
Biomass Secure Power	USA	Transesterification	Algae	Biodiesel
Bionavitas	USA	Light immersion technology	Algae	Biofuels

Blue Marble Energy	USA	Floatable pond (possible applications to wastewater treatment)	Cellulosic biomass, algae	Renewable energy feedstock, specialty biochemicals/ biogas/ biodiesel
Bodega Algae LLC	USA	Continuous-flow algae photobioreactors	Algae	Biofuels
Boeing	USA	N/A	Jatropha, algae	Aviation biofuels
British Airways	UK	Enzymatic hydrolysis	Biomass/algae	Ethanol/jetfuel
Cellana	Hawaii, USA	Photobioreactor/open pond	Marine microalgae	Algal bioproducts/biofuels
Chevron	USA	N/A	Algae/cellulosic biomass/wood, and swithgrass	Biofuels
Desert Sweet Biofuels	USA	Use agricultural waste for the growth of algae	Algae	Food, fuel, fertilizer, and feeds
Diversified Energy Corporation		Gasification, pyrolysis, combustion, anaerobic digestion, thermal-chemical, biotechnology-based, microwave	Algae	Ethanol, biodiesel, electricity, syngas, synthetic natural gas, hydrogen, and chemicals
Eco-Fuel Global	USA	N/A	Jatropha, microalgae, palm oil	Biofuels
Energy Derived	USA	Reverse algae diffusion (RAD) process	Algae	Biodiesel
ETC Green	USA	N/A	Jatropha, microalgae	Biodiesel, syndiesel
ExxonMobil	USA	N/A	Algae	Fuels, lubricants, petrochemicals, and algal oil
Greener Dawn Group	USA	Algae transesterification	Algae	Biodiesel
GreenFuel Technologies	USA	Cultivation of algae in natural setting by using the CO_2 emitted from a power plant	Algae	Jet fuel, ethanol, protein for foods, nutrients, pigments
INEOS	USA	Lipid and cellulose conversion	Algae	Biodiesel and ethanol
Ingrepo	UK	Grow algae in open pond by using industrial and agricultural waste waters	Algae biomass	Algae lipid
Inventure Chemical technology	USA	Patent pending algae-to-jet fuel product	Algae	Biofuel
Leaf Clean Energy	USA	Algae transesterification	Algae	Biodiesel
LiverFuels	USA	Open-water systems	Algal biomass	Fuels and other valuable co-products
Malaysian Rubber	Australia	Algae transesterification	Algae	Biodiesel
Neste Oil	UK	Open pond cultivation and NEXBTL renewable diesel production technology	Algae and vegetable oils	Biodiesel
OriginOil Inc.	USA	Open ponds	Algae	Diesel, gasoline, jet fuel, plastics, and solvents without the global warming effects

(continued)

Table 3 (continued)

Companies	Region	Technology (Process)	Feedstock	Primary product
PetroSun	USA	Open pond and closed reactor	Algae biomass	Biofuels
Piedmont Biofuels Coop	USA	Algae burning/transesterification	Algae	Biodiesel
Plankton Power	N/A	N/A	Algae	Biodiesel
Sapphire Energy	USA	Convert sunlight and CO_2 to renewable carbon-neutral alternatives to conventional fossil fuel	Genetically modified cyanobacteria, blue-green algae	Bio-crude oil
Seambiotic	Israel	Microalgal cultures in open ponds using flue gases like CO_2 and nitrogen from a nearby coal plant as feedstock	Algae cultivation	Food, fine chemicals, and biofuels
Shell Oil	USA	Algae transesterification	Algae	Biodiesel
Solazyme, Inc.	USA	Algae grown in dark in large tanks where they are fed sugar to supercharge their growth	Algae	Oil, biofuels, and green chemicals
Solena	USA	Patent plasma technology to gasify algae and other organic substance with high energy outputs	Algae	Biofuel
Solix Biofuels	USA	CO_2 produced during beer production used for the growth of algae	Algae	Biofuel
Storm Fisher Biogas	USA	Algae transesterification	Algae	Biodiesel
Sustainable Green Technologies, Inc.	USA	Microbe	Biomass waste/algae	Biodiesel
Synthetic Genomics, Inc.	USA	Algal cultivation	Algae	Algal fuel
TransAlgae Ltd.	Israel	Algae strains research	Algae	Biofuels
VG Energy Inc.	USA	Manipulating the metabolic functions of algae cells	Algae	Refinable oil and fuels
XL Renewables	USA	Thin-walled polyethylene tubing (Algae Biotape)	Algae	Protein, carbohydrates, ethanol, and biodiesel

location for algae farms. In these countries south of the Mediterranean, the climate is warmer, and the temperature does not drop below 15 °C throughout the year. This kind of climate is ideal for the growth of algae in open or closed pond systems. These systems would perhaps be the most suitable, efficient, and economically feasible for the growth of algae. Many countries in the Mediterranean basin have a large potential for harvesting algae. Some countries like Israel have begun to produce several strains for fuel production and have also been harvesting algae for medicinal purposes for decades. The southern countries bordering the Mediterranean Sea, such as Morocco, Algeria, Tunisia, and Egypt, are particularly attractive because of their high temperatures and huge unused desert land. In the future, Bio3 Dakhla, an algal industry in Dakhla, Morocco, anticipates investing billions on biofuel industries and are currently producing large amounts of *Spirulina* for human consumption. However, countries like Libya, Cyprus, and Turkey could also harvest algae on marginal land. Although these countries do not have sufficient water resources, they grow algae in recycled brackish or saline water since the algae is from a marine source and does not require fresh water for growth. Moreover, these countries are just developing and could strongly benefit from running these algal industries. Algae farming can offer jobs for local people, and the transfer of technologies to developing countries can only be beneficial (Piccolo 2009; Singh and Gu 2010).

A complete survey has been carried out for the various aspects of algal cultivation (Edward 2009), including the profiles of various companies involved in the growth of algae for biofuel as well as other applications around the world. The classification is based on various algae farming technologies being used. The reported information has been summarized in Table 3. Some of these companies are exploring suitable regions for algae cultivation, but only a few are using open pond systems or natural settings. Initially, closed systems or photobioreactors (PBRs) were proposed to cultivate algae; these bioreactors are installed near a source of CO_2, and thus serve an additional purpose of carbon sequestration. However, natural settings have the least capital cost. So regions in which enough land is available to grow algae in open ponds without interfering with the food chain are an attractive option.

9 Cultivation of Microalgae

Cultivation is the biggest part of generating biomass from microalgae. This has been done on an industrial scale for many years with the help of solar energy (photoautotrophically) and is economically feasible for large-scale production for other uses (Borowitzka 1997). There are two main cultivation systems: open pond (raceways) and closed systems (PBRs). Each system is influenced by intrinsic properties that play a major role in feasibility, including strains used, nutrients, costs of land and water, downstream process, manpower, and climatic conditions (Borowitzka 1992).

9.1 Open Pond System

The open pond production system has been used to cultivate algae since the 1950s (Borowitzka 1999). These systems can be divided into natural waters (lakes, lagoons, and ponds) and artificial systems or containers (Jiménez et al. 2003). The most commonly used cultivation system is raceway ponds. They are typically made up of closed, loop, oval-shaped recirculation channels (Fig. 1f). They are generally constructed in concrete (Fig. 2b), but plastic-covered earth-lined ponds have also been used (Brennan and Owende 2010). The plastic culture system is cheaper, easy to operate, and more durable than closed systems (Zeng et al. 2011; Rawat et al. 2011). Most paddle wheel-driven raceway ponds are 60 cm in depth, and the paddle wheels are helpful to mix and circulate the culture, thus reducing the shading effect required to stabilize algae growth and productivity (Chisti 2007; Brennan and Owende; 2010; Norsker et al. 2011). In continuous production, algae broth and nutrients are introduced near the paddle wheel to prevent sedimentation and can be circulated through the loop to the

Fig. 2 (**a**) An R&D type of photobioreactor, (**b**) concrete raceway pond with a paddle wheel, (**c**) dissolved air flotation (DAF) tank, (**d**) culture dryer (industrial scale), (**e**) flocculated culture

harvest point. However, ponds can have various advantages and limitations. The major limitation is that productivity is lower than closed systems, and environmental factors cannot be controlled (Chisti 2007; Borowitzka 1999; Zeng et al. 2011). Generally, ponds are more susceptible to weather conditions, evaporation, temperature, and lighting. Increases in the culture density of CO_2 transfer rate and light limitations could slow down the cell growth of microalgae.

Atmospheric CO_2 is used to fulfill the carbon requirement, but it contains only 0.03–0.06 %, which is not enough for the growth of microalgae. To improve the overall biomass productivity, submerged aerators may be installed to enhance CO_2 absorption, these will be helpful as the proper mixing of culture can minimize the impact of both CO_2 and light limitations, thus improving biomass productivity (Brennan and Owende 2010). Open pond systems occupy more land area, and contamination with other algae, bacteria, and protozoans can reduce microalgae growth (Blanco et al. 2007; Brennan and Owende 2010; Rawat et al. 2013). The selection of land and water availability for microalgae culture is another important factor. Usage of marginal and non-arable land has more advantages than the use of arable land. Maintenance and cleaning of open systems is easier and requires less energy input than PBRs (Ugwu et al. 2008; Brennan and

Owende 2010; Rawat et al. 2013) and therefore have the potential for a large net energy production (Rodolfi et al. 2009). *D. salina* is one of the most commonly cultivated strains in open pond systems, and, during 2008, the unit price was about €2.55/kg of dry biomass, which was considered too high to justify production for biofuels (Tan 2008; Brennan and Owende 2010; Rawat et al. 2013).

9.2 Closed System

The closed system is designed to overcome all the problems associated with open ponds (contaminations and expected biomass). PBRs are continuous culture systems that can achieve expected biomasses of up to 6.7 g/L (Chisti 2007; Ranjbar et al. 2008; Bai et al. 2011). This technology is used to grow microalgae for the production of high-value compounds such as pharmaceuticals, neutraceuticals, and cosmetics that cannot be grown as a monoculture in open pond systems. Using PBRs, single species of microalgae can be grown for long durations with minimal contamination risk. PBRs have lower direct exchanges of gases and contaminations (e.g. microorganisms, dust) than open pond systems. Different PBR models (indoor and outdoor) have been developed, including tubular, flat plate, airlift, bubble column, and stirred tank (Xu et al. 2009). The principle behind the PBR is reduction of the light path, thereby increasing the amount of light received by each cell (Borowitzka 1999). The culture is generally mixed by airlift or mechanical stirring/pumping. Mixing of culture is important for gaseous exchange within the system (Brennan and Owende 2010; Rawat et al. 2013). High biomass yield, which depend on good control of culture parameters such as temperature, pH, and CO_2 concentration, etc., can be achieved using closed PBRs (Suh and Lee 2003), but the capital costs are ten times higher than those of open ponds (Carvalho et al. 2006). However, the combination of both can be profitable because microalgae can be grown in open ponds while reducing contamination by undesired species (Huntley and Redalje 2008). In this culture process, the first step of microalgae production is controlled by temperature (e.g. sea water bath [16–18 °C]) PBR. Microalgae are transferred into an open pond for 5 days in a second culture step (Huntley and Redalje 2006, 2008). Closed PBRs have the advantage of high productivity, low contamination risk, efficient CO_2 capture, continuous runs, and controlled growth conditions. The major drawbacks are the higher capital investment and operating costs.

9.2.1 Tubular Photobioreactor

The tubular PBR is one of the most suitable bioreactors for large-scale outdoor cultivations since they expose a large surface to sunlight. A tubular reactor consists of vertical, horizontal, inclined, or helix-shaped tubes connected with a pipe system (Molina et al. 2001; Ugwu et al. 2002; Brennan and Owende 2010). The algae-suspended fluid is able to circulate in this tubing. The tubes are generally of transparent plastics or borosilicate glass and the circulation is kept constant by a pump at the end of the system. The diameter of the tube is 0.1 m or less, and helpful for the high penetration of light into the dense culture (Chisti 2007). Either a mechanical pump or an airlift system is used to recirculate the algal cultures after allowing CO_2 and O_2 to be exchanged between the liquid medium and aeration gas as well as providing a mechanism for mixing (Eriksen 2008). To encourage gas exchange in the tubes, agitation and proper mixing is very important. The introduction of gas takes place at the end or beginning of the tube system. This method of introducing gas causes deficiency in CO_2, a high concentration of oxygen at the end of the unit during circulation, and poor efficiency. Therefore, cultures are generally reticulated by pump, passing through a degasser at regular intervals in order to remove excess oxygen. The largest closed PBRs are tubular (e.g. the 25 m^3 plants at Mera Pharmaceuticals, Hawaii [Olaizola 2000] and the 700 m^3 plant in Klötze, Germany [Pulz 2001]). Tubular reactors are currently being used for the culture of high-value products such as astaxanthin (Fig 2a).

9.2.2 Bubble Column Photobioreactor

A bubble column PBR consists of a vertically arranged cylindrical column made of transparent material (Eriksen 2008). This PBR has the highest volumetric mass transfer rates, proper mixing, and controllable growth conditions (Eriksen 2008). The cost of this bioreactor is very low, and it is compact and easy to operate. The introduction of gas takes place at the bottom of the column and causes a turbulent stream to enable optimum gas exchange. At present, these types of reactors are built with a maximum diameter of 20–30 cm in order to ensure the required supply of sunlight. This type of PBR allows a reduction of harmful shear forces.

9.3 Two-Step Cultivation System

The two-step cultivation system involves a combination of raceway and PBR designs. This system has been traditionally used to develop inoculum for aquaculture operations. The main advantage of this system is the production of inoculum that is free from contamination (Schenk et al. 2008). The first step is the fast cultivation of biomass in the PBR; the second step is stress cultivation in open ponds. The first step allows for better protection of the growing biomass in the PBR during the early stages, and CO_2 capture is maximized. The microalgae suspension is transferred in open ponds that have enough nutrients, are low in nitrogen, and maintain high CO_2. The second step in open raceways has few problems because a high algal density is more resistant to contamination, and this phase is nutrient depleted, avoiding the growth of contaminating species. The combination of PBR and an open pond has proven efficiency for astaxanthin production (Huntley and Redalje 2006). It is currently being used by companies that are developing biofuel applications. Hybrid systems can produce as much as 20–30 tonne ha^{-1} of lipids annually, depending on suitable climatic conditions (Rodolfi et al. 2009).

10 Wastewater as a Source of Nutrients for the Growth of Microalgae

Wastewater is an excellent, cheap, and readily available medium for the growth of various microalgal strains (Schenk et al. 2008). Wastewater also contains macronutrients that help in the growth of microalgae (Raja et al. 2004; Hosikian et al. 2010; Rawat et al. 2011). The macronutrients present in wastewater are ammonia, nitrate, phosphate, urea, trace elements such as vitamins (biotin and thiamine), certain trace metals, and, rarely, radioisotopes. The growth of several microalgae is found to be suitable in wastewater because of the pH and dissolved CO_2 concentration. Large-scale production of microalgae for biofuels using wastewater has the possibility to improve the economics of biomass production. Growth of microalgae using wastewater also has some disadvantages, such as bacterial and viral contamination that may negatively affect the biomass and production process. Use of wastewater for the growth of microalgae might lead to an adequate cleaning of the culture system (Lam and Lee 2012) and also help to reduce the eutrophication in the aquatic environment. Microalgae can also be used for the removal of rich nutrients available in wastewater (Raja et al. 2008) (e.g. *C. vulgaris* is used for the removal of nitrogen and phosphorous from wastewater, with an average removal efficiency of nitrogen [72 %] and phosphorus [28 %]; 3–8 mg/LNH^{4+} and 1.5–3.5 mg/LPO_4^{-3}) (Aslan and Kapdan 2006). Other microalgae cultures used for the removal of nutrients are *Chlorella*, *Scenedesmus*, *Spirulina* spp., *Nannochloris*, *B. brauinii*, and cyanobacterium *Phormidium bohneri* (Lee and Lee 2001; Olguín et al. 2003; An et al. 2003; Jimenez-Pérez et al. 2004). Some companies actively involved in culturing algae using wastewater for biofuel production include Algae wheel Technologies (USA), Algal Scientific Corporation (USA), Aquaflow Binomic Corporation (New Zealand), Blue Marble Energy (USA), Community Fuels (USA), Nanoforce

Incorporates (USA), and Western Michigan University (USA).

11 Harvesting Microalgal Biomass

Harvesting of microalgal biomass is one of the important steps in producing maximum biomass. It can actually contribute 20–30 % of total biomass production cost (Chisti 2007). The small size of microalgal cells (typically ranging from 2 to 200 μm) makes the harvesting process very difficult. There are a few techniques in the harvesting of microalgae, including centrifugation, flocculation, filtration and screening, gravity sedimentation, flotation, and electrophoresis techniques (Uduman et al. 2010; Brennan and Owende 2010). The selection of harvesting techniques is dependent on the physical properties of microalgae such as size, density of the slurry, intracellular biomass composition, and yield of desired products (Brennan and Owende 2010). We summarize some of the techniques that are currently being used for the recovery of microalgal biomass.

11.1 Filtration

Conventional filtration may be inadequate for biomass recovery and is most appropriate for the harvesting of large (>70 μm) microalgae such as *Coelastrum proboscideum* and *Spirulina platensis*. The small (<30 μm) microalgae like *Chlorella*, *Dunaliella*, and *Scenedesmus* cannot be harvested using this technique (Mohn 1980). For recovery of smaller algal cells (<30 μm), membrane microfiltration and ultra-filtration are a technically feasible alternative to conventional filtration. Microfiltration and ultra-filtration are a form of membrane filtration using hydrostatic pressure. Microfiltration is one of the most efficient and suitable methods for harvesting fragile algal cells (Grima et al. 2003; Mata et al. 2010). Conventional filtration works under pressure or suction; filtration aids such as diatomaceous earth or cellulose can be used to increase efficiency. This method has demonstrated that the filtration process is moderately effective for large microalgae, whereby a chamber filter press can achieve a concentration factor 245 times the original concentration of *C. proboscideum*, to produce sludge with 27 % solids. The disadvantages of membrane filtration include the sporadic requirement for membrane replacement and elevated production costs due to energy-intensive pumping. It is more cost effective than the centrifugation process for the processing of low-culture volume (<2 m^3/day). Due to the cost of membrane replacement and pumping in large-scale production (>20 m^3/day), centrifugation may be one of the most economic methods for harvesting microalgae (MacKay and Salusbury 1988).

11.2 Centrifugation

Centrifugation is a method of separating algae from the medium using a centrifuge to cause the algae to settle in the bottom of a flask or tank. It is a useful device for both biolipid extraction from algae and chemical separation in biodiesel. The centrifuge works using the sedimentation principle, where centripetal acceleration is used to evenly distribute substances (presents in a solution for small-scale applications) of greater and lesser density. Centrifugation is currently considered too expensive and to increase production costs due to rising power consumption (Grima et al. 2003). This method is preferred for harvesting algal biomass especially for producing prolonged shelf-life concentrates for aquaculture usage. The main advantage of this technique is that 95 % of the cells are efficiently harvested at 13,000 × *g* by increasing the gravitation field (Greenwell et al. 2010) thus it increases the slurry concentration up to 150 times more and 15 % total suspended solids are technically possible to harvest (Mohn 1980). It has certain disadvantages, which include the high energy costs and potentially higher maintenance requirements (Bosma et al. 2003).

11.3 Gravity Sedimentation

Gravity sedimentation is commonly applied and is a rapid intensive method for separating microalgal biomass from huge volumes of water (or wastewater). Gravity sedimentation methods are based on Stokes's Law (Schenk et al. 2008) (i.e. settling characteristics of suspended solids are determined by the density and radius of algae cells and induced sedimentation velocity). Higher microalgal biomass can be achieved by sedimentation using lamella separators and sedimentation tanks. This method is only suitable for harvesting of large microalgae (ca. >70 µm) such as *Spirulina* (Muñoz and Guieysse 2006). The success of solid removal via the gravity settling method depends on the density of the microalgal particles. Low-density microalgal particles do not settle well and are ineffectively separated by settling (Edzwald 1993). This technique has some advantages: it is cost effective, and it is possible to recycle water because no potentially toxic chemicals are used.

11.4 Flocculation

An integrated approach is needed to minimize the energy consumption of harvesting microalgae (Benemann et al. 1977). Several harvesting methods have shown that flocculation combined with flotation or sedimentation and followed further by dewatering with centrifugation or filtration is the most promising and cost-effective method (Schenk et al. 2008). This is the bulk-harvesting technique whereby the dispersed microalgal cells aggregate and form larger particles with a higher sedimentation rate. Microalgal cells are negatively charged, which prevents aggregation in aqueous suspension, so multivalent cations or cationic polymers are useful for the neutralization of microalgal cells. Flocculation can be induced in different ways. Induced chemical flocculation using multivalent metal salts like aluminum sulphate ($Al_2(SO_4)_3$), ferric chloride ($FeCl_3$), ferric sulphate ($Fe_2(SO_4)_3$), or other chemical flocculants has been studied extensively (McGarry 1970; Lee et al. 1998; Papazi et al. 2010) and some of these are applied in industry. Aluminium sulphate is an effective coagulant being widely used in flocculating algal biomass (*Scenedesmus* and *Chlorella*) in the wastewater treatment process (Grima et al. 2003).

Although an easy and effective method, it is not feasible and sustainable for large-scale harvesting of microalgae because microalgae production plants have excess cationic flocculent that needs to be removed from the medium before it can be re-used, thus leading to extra operational costs (Schenk et al. 2008). Flocculation can also be induced by changing the culture conditions with the application of extreme pH, nutrient depletion, changes in temperature dissolved O_2 level. The pH of the algal culture is adjusted to 10–10.6 using NaOH, followed by the addition of non-ionic polymer Magnfloc LT-25. This method of harvesting microalgal biomass leads to a concentration of 6–7 g L^{-1} (Knuckey et al. 2006). The process has been successfully applied to a variety of species, with flocculation efficiency of >80 %. The other method that has been proposed for flocculation of microalgae is biologically induced, where bacteria have been applied successfully in wastewater treatment (Lee et al. 2009). The disadvantage of this method is that it leads to undesirable bacterial contamination of the algal production plant. In recent years, naturally flocculating diatom *Skeletonema* has been used to form flocs of *Nannochloropsis* (Schenk et al. 2008). As diatoms have a silica-based cell wall, they require different medium composition than most microalgal strains used for biodiesel production, which leads to additional cultivation costs. Chitosan is another bio-flocculant, and this method is very sensitive to pH. For freshwater microalgae, the maximum flocculation was obtained at pH7.0, whereas, for marine species, it is low (Divakaran and Pillai 2002). The remaining water can be reused for the cultivation of algae. Heasman et al. (2000) used chitosan as a flocculant for *Tetraselmis chui*, *Thalassiosira pseudonana*, and *Isochrysis* sp. To complete the flocculation process of these strains, 40 mg/L of chitosan is required. On the other hand, 150 mg/L of chitosan was found to complete the flocculation process of *Chaetoceros muellaris* (Heasman et al. 2000). Although chemical flocculation is often reported to incur fewer operating costs to harvest algae, this method leads to significant pollution of the environment (Li et al. 2008a). The interruption of the CO_2 supply in the algal system can cause algae to flocculate on its own, termed 'autoflocculation'. In most cases, this phenomenon is associated with high pH due to photosynthetic CO_2 consumption corresponding with precipitation of magnesium, calcium, phosphate, and carbonate salts with algae. Where calcium phosphate is used, excess calcium ions (positively charged) tend to react with algae (negatively charged) and they bind together to provide an autoflocculation process (Sukenik and Shelef 1984). Figure 2e shows the flocculating agents added in to the algal culture and starting to coagulate algae cells.

11.5 Flotation

Flotation is a method used in combination with flocculation to harvest algae. It is a simple method by which algae can be made to float on the surface of the medium. Due to the increase in the lipid content of the cells, some strains may float naturally (Bruton et al. 2009). The main advantage of this method is that it is cheap and it can be used to harvest large-scale microalgae. A few disadvantages of this method are that the flocculating agents may lead to contamination, and evidence of its technical viability is also very limited (Grima et al. 2003). There are two types of flotations:

11.5.1 Dissolved Air Flotation (DAF)

Dissolved air flotation (DAF) separates algae from its culture by using both froth flotation and flocculation (Fig. 2c). For the flocculation of microalgal cells, alum is used to increase the floc size before applying DAF, fine bubbles are supplied by an air compressor. Alum is a common name for several trivalent sulfates of metal such as aluminum, chromium, or iron and a univalent metal such as potassium or sodium (e.g., $AlK(SO_4)_2$).

11.5.2 Froth Flotation

This is another method of separating algae from the medium by adjusting the pH and bubbling air through a column to create a froth of algae. The algae collect in foam above the liquid level and can be separated by suction. The pH may vary depending on the algal species. The major disadvantage of the froth flotation is that it is not economically feasible.

12 Dehydration of Algal Biomass

After algal biomass is harvested, it must be processed quickly. Depending on the final product, algal biomass can be processed by dehydration or drying (Fig. 2d). Several methods area available to process algal biomass, namely, solar drying, low-pressure-shelf drying, drum drying (Prakash et al. 1997), spray drying (Desmorieux and Decaen 2005), fluidized bed drying (Leach et al. 1998), freeze drying (Grima et al. 1994), and refractance window dehydration technology (Nindo and Tang 2007). When compared with other dehydration processes, solar drying is the cheapest. However, some of the major disadvantages are that solar drying requires a long time and a large space, and there is a risk of material loss (Prakash et al. 1997).

13 Lipid Extraction

Most microalgae accumulate neutral lipids; due to the low degree of unsaturation, these lipids are essential for biodiesel production. There are several methods for extracting lipids from microalgae, namely, the Folch method, the gravimetric method, and the Bligh and Dyer method. Solvent extraction (n-hexane) and gravimetric determination are the two methods often used by researchers for the extraction of lipids from the microalgal cell with or without cell disruption (Viswanath et al. 2010). To identify and quantify microalgal lipids, other analytical methods such as thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas-chromatography mass-spectrometry (GC-MS) are used (Elsey et al. 2007). The choice of method depends on the efficiency, accuracy, and cost effectiveness, ease of use, high throughput capability, robustness, exactness, and reproducibility (Viswanath et al. 2010). Screening of a large number of lipid-producing microalgal samples under laboratory conditions is difficult and time consuming. Hence, to overcome this problem, attention is increasingly focused on in situ measurement of lipid content, such as Nile Red-NR staining (Fig. 1e), time-domain nuclear magnetic resonance (TD-NMR), and boron-dipyrromethene (Bodipy) (Mutanda et al. 2011).

13.1 Extraction Technique

In order to produce biodiesel from microalgae, several methods can be used for the extraction of biofuel and high-value products. The important lipid-extraction techniques are chemical solvents, supercritical CO_2, physico-chemical, biochemical and direct trans-esterification.

13.2 Solvent Extraction

Microalgal oil can be extracted using chemicals such as n-hexane, chloroform, benzene, diethyl ether, and ethanol. In large-scale studies, Yaguchi et al. (1997) used choloroform-methanol blends for the extraction of lipid and obtained high lipid yields of up to 83 % (g lipid/g dry weight). n-Hexane is the most commonly used solvent for the extraction of lipids due to its lower toxicity and its affinity for non-lipid contaminants (Halim et al. 2011). For example, Miao and Wu (2006) used hexane as a solvent for the extraction of lipids from *Ch. Protothecoides*; the yield of lipid contents was up to 55 % (g lipid/g dry weight). Soxhlet extraction is not used at industrial scales due to a high energy requirement (Halim et al. 2011). Ethanol, octonol, or 1,8-diazabicyclo-(5.4.0)-undec-7-ene (DBU) yield lower FAME than n-hexane extraction (Samorì et al. 2010). The advantage of using solvents for lipid extraction is that they are inexpensive and very efficient for oil extraction. For the separation of other valuable products, such as beta-carotene, astaxanthin, and other essential fatty acids, from microalgae, solvent extraction is used extensively (Grima et al. 2003).

13.3 Supercritical CO_2 Extraction

Supercritical fluid extraction is one of the most effective methods for the extraction of oil from microalgae (Mendes et al. 1995). CO_2 is first heated and compressed until it reaches liquid-gas state or above its critical point. Harvested microalgae are added to act as a solvent. Limited methods have been studied for the recovery of lipids from microalgae and to transform them to biodiesel (Halim et al. 2011). Certain studies recovered lipid content of up to 26 % (g lipid/g dry weight) from *Nannocloropsis* sp. (Andrich et al. 2005). Halim et al. (2011) used supercritical CO_2 extraction at 60 °C, and pressure of 30 MPa, to extract lipids from *Chlorococcum* sp. and obtained higher lipid contents of 5.8 % g lipid/g dry weight, whereas using hexane Soxhlet extraction obtained only 3.2 % g lipid/g dry weight. Using wet algae obtained a maximum lipid yield of 7.1 % g lipid/g dry weight, which was lower than other *Botryococcus* species, as the lipid is high 28.6 % g lipid/g dry weight (Lee et al. 2010). This indicates that the supercritical CO_2 lipid extraction was enhanced in the presence of water with the blend of microalgae. The main advantage of this method is that it is not toxic, is easy to recover, is usable at low temperatures, and is relatively rapid because of the low viscosities and high diffusivities (Andrich et al. 2005). The disadvantage is that it requires expensive equipment (Perrut 2000) and a huge amount of energy to reach high pressure (Tan and Lee 2011).

13.4 Physico-Chemical Extraction

The physico-chemical technique can be used for the disruption of cells in order to extract lipids from microalgae (Cooney et al. 2009; Lee et al. 2010). Some of the physico-chemical techniques are microwave, autoclaving, osmotic shock, bead-beating, homogenization, freeze-drying, French press, grinding, and sonication. Microwave and bead-beating methods yield a higher lipid content from microalgal cells. In *Botryococcus* sp., using 5 min microwave pre-treatment increased the lipid extraction yield from 7.7 to 28.6 % g lipid/g dry weight (Lee et al. 2010).

13.5 Biochemical Extraction

Only limited studies have used biochemical extraction to extract lipids from microalgae. For example, after 72 h cellulase hydrolysis pre-treatment, 70 % of sugar was obtained, whereas the lipid content was increased slightly from 52 to 54 % g lipid/g dry weight in *Chlorella* sp. (Fu et al. 2010).

13.6 Direct Transesterification

Direct transesterification is a process that mixes alcohol and a catalyst with microalgae without prior extraction. In microalgae, many catalysts have been examined, including hydrochloric (HCl) or sulphuric acid (H_2SO_4), but acetyl chloride (CH_3COCl) produces a higher FAME yield of 56 % g FAME/g dry weight (Cooney et al. 2009). This process can be enhanced by coupling it with microwave heating. The heterogeneous catalyst (SrO) together with heating with microwaves increased the FAME yield from 7 to 37 % g FAME/g dry weight in *Nannochloropsis* (Koberg et al. 2011). The disadvantage of this technique is the high harvesting cost due to the necessity for dry microalgal biomass. The overall flow chart of the processing of algal biomass to biodiesel is shown in the Fig. 3.

14 Conclusion

Increases in atmospheric CO_2, and depletion of petro-diesel and mineral oil reserves requires alternative environmentally friendly energy sources. To overcome this problem, production of biodiesel from microalgal biomass might be a solution to reduce CO_2 emissions from industry and could also meet the global demand for transport fuels. Production of biodiesel from microalgae is an emerging technology and an economical choice because of its availability and low cost. Microalgae have several advantages over conventional crops in that less land is required and non-arable land can be cultivated; microalgae double their weight with respect to biomass within 24 h; they require low pesticide application and have no impact on food security; and microalgae biomass can also be used in other energy-generation processes after the oil has been extracted. Many companies are already achieving commercial-scale production of microalgal biofuels. Large-scale cultivation and harvesting systems are needed for the production of algal biodiesel to reduce the cost per unit area. Large-scale microalgal biomass can be achieved using open pond or PBRs, and each system has its own advantages and disadvantages. The open pond system is widely used for large-scale production due to its advantages and the potential for CO_2 sequestration when compared with PBRs. Some essential factors need to be optimized for large-scale application, including strain selection, seed culture preparation, medium composition, biomass and lipid yield optimization, harvesting, and extraction of lipids from biomass. Certain other important factors include providing optimum conditions of certain parameters such as light, nutrients, temperature, and CO_2 and O_2 levels. However, the above parameters cannot be controlled in an open pond system. Due to the high costs involved in the harvesting and extracting of lipids from the microalgal biomass, more effort must be made to reduce process costs and to increase the quality of biodiesel.

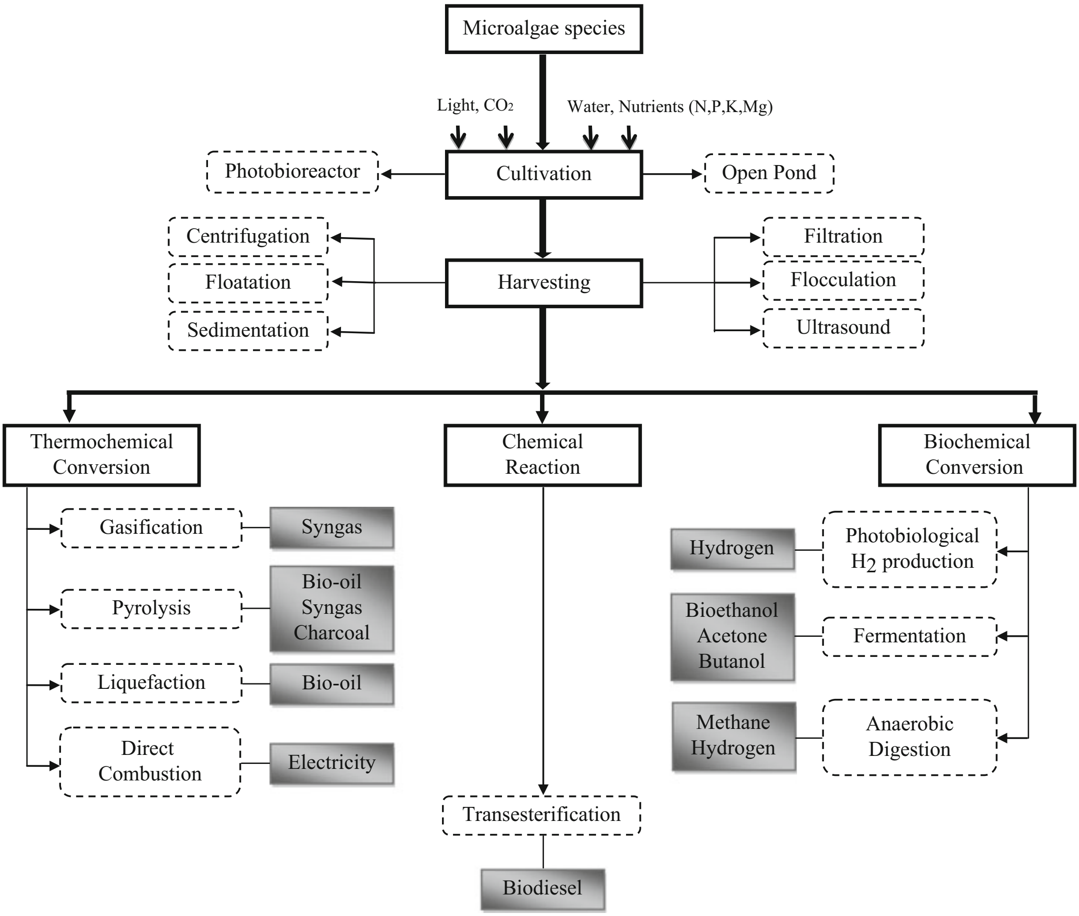


Fig. 3 Process of turning potential algal biomass into biofuel

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Advancement and Challenges in Harvesting Techniques for Recovery of Microalgae Biomass

Amrita Difusa, K. Mohanty, and V.V. Goud

Abstract

Microalgae have been known for their potential in the development and production of sustainable biofuel and the mitigation of CO_2 generation. The process of biofuel production from microalgae involves various technologies, from cultivation systems to harvesting of microalgal biomass and oil extraction. The harvesting of microalgae biomass has been the bottleneck of the whole system. This review presents recent advances in harvesting technology and efficiency in terms of biomass recovery and processing further downstream. The different harvesting techniques and their challenges are compared and discussed. This review focuses on recent technologies and provides information on further development of commercially viable technology for the production of microalgae-derived biofuel.

Keywords

Microalgae • Harvesting • Biofuel • Cultivation

1 Introduction

The growing energy demand has turned the world's attention towards the development of clean and sustainable energy sources. In comparison with various renewable energy sources, biofuels are estimated to participate in the near future for their extensive potential in the global energy infrastructure (Chen et al. 2011). Generations of biofuel feedstocks have arrived; with their potential outcomes also come some disadvantages. Microalgae have been considered an attractive biomass feedstock for biofuel production because of its extremely rapid growth and rich oil content (Chisti 2007). Owing to its multi-potentiality, microalgae can be cultivated and harvested throughout the year in arid regions using water that is unsuitable for agricultural purposes (Uduman et al. 2010). Indeed, the use of potable water can also be minimized by incorporating

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microalgae cultivation with wastewater management. Unlike other energy crops, microalgae require a minimal area of cultivation to meet the growing demand for energy. In fact, only 1–3 % of the total US crop area would be required to produce an algal biomass that would satisfy 50 % of transport needs in the USA (Uduman et al. 2010). In contrast, India requires only 2 % of its geographical area to produce 200 billion gallons of biodiesel for transportation purposes (Khan et al. 2009). This feedstock has been favored due to its high oil content. Almost all microalgae species are able to accumulate up to 20–50 % of oil/lipid per dry weight and, depending on cultivation conditions, some species can produce up to 50–70 % of oil/lipid per dry weight (Chisti 2007). With its tremendous oil-producing capacity, microalgae feedstock has involved a high operational cost in downstream processing, particularly in the harvesting step, which has restricted the large-scale production of microalgae-derived biofuel. Microalgae harvesting technologies have been extensively performed for biofuel production. The maximum dewatering of microalgae culture is required to simplify the next step, lipid extraction, and the dilute nature of microalgal culture mean that the processing steps require substantial energy and thus add high operational costs to the overall process. Thus, biomass harvesting has been the major challenge of downstream processing. This has initiated a desire for efficient harvesting technologies with maximum recovery of biomass (Uduman et al. 2010). Various harvesting techniques for biomass recovery include centrifugation, flocculation, filtration and screening, gravity sedimentation, flotation, and electrophoresis techniques (Chen et al. 2011). All these methods have advantages and disadvantages. Some harvesting methods are lacking due to high costs, intensive energy requirements, and flocculent toxicity, etc. These drawbacks have initiated to develop an efficient and economically viable technique for harvesting of microalgae.

2 Microalgae Harvesting Processes and Challenges

Harvesting of microalgae biomass involves various techniques that require one or more solid–liquid separation steps. The processes used for the recovery of microalgae have significant problems in separating the algal cells from water because of the typical cell size ranges from 0.2 to 30 μm and require large volumes of dilute culture (<0.5 kg m^{-3} dry biomass) to be handled to recover the biomass. Biomass recovery has been estimated to contribute about 20–30 % to the total cost of biomass production (Molina Grima et al. 2003). The harvesting steps of the system are not only costly but also affect the downstream processes. However, biomass recovery is an essential part of the system and needs to be performed for further product processing. Lowering the cost of algal biomass recovery with an appropriate harvesting technique that will allow the recovery of viable biomass remains a challenge.

Any suitable harvesting method must be applicable to a wide range of microalgae strains cultivated in different cultivation techniques. Filtration is the most commonly used method. However, centrifugation is used for recovery of high-value products. It is a very rapid technique and can process large volumes with highly efficient biomass recovery. In contrast, for recovery of low-value product, gravity sedimentation preceded by flocculation may be a good option. Gravity sedimentation or dissolved air flotation (DAF) is a common approach for biomass recovery from sewage-based processes (Molina Grima et al. 2003). Hence, it is very important to understand the various technologies involved in harvesting in order to maximize the efficiency of biomass recovery at minimum expense. The recovery of microalgae cells has been differentiated in different types of harvesting processes shown in Fig. 1.

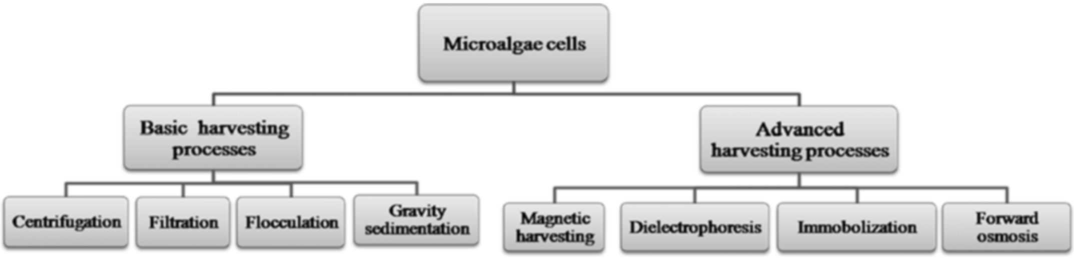

Fig. 1 Different types of microalgae harvesting process

2.1 Centrifugation

Centrifugation as a method for harvesting microalgae is preferred over other harvesting techniques such as DAF and drum filtration (Harun et al. 2010). The process is efficient, with biomass recovery of 80–90 % within 2–5 min (Chen et al. 2011). Heasman et al. (2000) reported that centrifugation of algal cells at 13,000 rpm provides maximum harvesting efficiency of 95–100 % and cell viability of 88–100 %, whereas its efficiency decreases to 60 % at 6,000 rpm and 40 % at 1,300 rpm. The method is suitable for laboratory-scale operation when the culture concentration is above 30 mg L^{-1}. The method of centrifugation and cell viability depend significantly on the algal species. Basically, the centrifugation process is reliable for recovery of metabolites (Harun et al. 2010). However, recovery of algal biomass under a rotational velocity of 3,000 rpm is evident, with 84 % removal efficiency of 0.2 g L^{-1} algal cultures at a flow of 100 gal min^{-1} (Dassey and Theegala 2013). The harvesting of microalgae cells is accompanied by the drying process and it responds effectively when the processed algal biomass is increased to at least 20 % dry weight during the dewatering stage (Dassey and Theegala 2013). Although the processes are rapid, they are also very energy intensive. The technique involves high gravitational and shear forces, which can damage cell structure. Large-scale harvesting is also a great challenge in terms of time and cost (Chen et al. 2011).

2.2 Filtration

Filtration is one of the simplest traditional methods of separating the algal cells, but with the advancement of modern techniques it has become the most competitive method compared with other harvesting options. The filtration process can range from simple screening or micro-strainers to dewatering up to complex vacuum or pressure filtration systems. There are many different forms of filtration, such as dead end filtration, microfiltration, ultra filtration, pressure filtration, vacuum filtration, and tangential flow filtration. This process involves separation of a suspension through a permeable medium, retaining the algae while passing through the filter (Harun et al. 2010). Filtration processes operating under pressure are more efficient for recovering large microalgae, while it is not suitable for the recovery of algae species such as *Scenedesmus*, *Dunaliella*, and *Chlorella* due to their small dimensions. Various filtration mediums have been studied for the recovery of small-celled *Dunaliella* but have been found to be impractical. In contrast, filtration through diatomaceous earth has proved exceptional (Uduman et al. 2010). For appropriate microalgae dewatering, different makes and brands of pressure and vacuum filtration units have been studied and the pressure belt filter and vacuum filter thickener have been found to be inappropriate for harvesting *C. proboscideum.* However, tangential flow filtration (TFF) is one of the high-rate microalgae harvesting methods and is reported to recover about 70–89 % of biomass (Chen et al. 2011). Membrane technology for recovering algal biomass is also a possible alternative to conventional filtration. Membrane technology is usually used to recover algal cells from small aquaculture farms for feeding shellfish larvae. However, the same technique is inefficient for harvesting algal biomass from large-scale cultivation processes (Molina Grima et al. 2003). Membrane technology also has a problem with fouling due to the formation of an algal layer causing pore blockages and a decrease in permeability (Ladner et al. 2010). However, frequent backwash of the membrane helps to control this fouling. The polyvinylchloride (PVC) ultrafiltration (UF) membrane employed with air-assisted backwash with air scour for harvesting *Scenedesmus quadricauda* showed efficiency and average permeability of 46.01 g $(m^2\ h)^{-1}$ and 45.50 l $(m^2\ h)^{-1}$, respectively (Zhang et al. 2010). Understanding the drawbacks of the higher efficacy and decreasing the fouling of membrane filtration processes remains a challenge. In terms of economic feasibility, membrane technologies are not cost effective for large-scale harvesting of algal biomass. However, harvesting small volumes of algal biomass with microfiltration (e.g., <2 $m^3\ day^{-1}$) is more cost effective than centrifugation (Molina Grima et al. 2003).

2.3 Flocculation

Flocculation is considered an effective process for large-scale harvesting of microalgae culture (Wu et al. 2012). The process is based on the negative surface charge carried by the microalgal cells that prevents self aggregation in suspension. The surface charge can be countered by the addition of flocculants such as multivalent cations and cationic polymers to amass the biomass from the culture (Harun et al. 2010). Flocculants must be inexpensive and non-toxic to the culture. In addition, the flocculent should not have any adverse effect on further downstream processing. Multivalent metal salts such as ferric chloride ($FeCl_3$), aluminum sulfate ($Al_2\ (SO_4)_3$, alum), and ferric sulfate ($Fe_2\ (SO_4)_3$) are widely used for flocculation (Molina Grima et al. 2003). Microalgae also have a tendency for self-flocculation, known as auto-flocculation. The process is sensitive to pH and it increases with the consumption of dissolved carbon dioxide. At super saturation of calcium and phosphate ions, the calcium phosphate precipitate will be positively charged. Thereby algae cells which are negatively charged serve as a solid support for the precipitant and charge neutralization is accomplished. A pH range of 8.5–9.0 was found to be optimum for auto-flocculation (Christenson and Sims 2011).

Polyelectrolytes are cationic polymers that are also used as an alternative flocculent. These are found to be better than the non-polymerized metal flocculants and are effective over a wide range of pH conditions (Molina Grima et al. 2003). Polymer flocculants induce the physical linkage of the cells via bridging. Ideally, the polymer should have high molecular weight and charge to enhance their binding capabilities. Tenney et al. (1969) also studied cationic and anionic polyelectrolytes for harvesting *Chlorella* species. Recovery of *Chlorella* cells with cationic polymers showed better results than anionic polyelectrolyte. Although cationic polymers are effective for harvesting freshwater microalgae at a minimum concentration of 1–10 mg ml^{-1}, they can inhibit the process in a high saline environment (Molina Grima et al. 2003). According to reports, the flocculation efficiency of marine algae decreases with an increase in the ionic strength of the media up to 36 g L^{-1} salinity. In this regard, the optimal dosage for flocculating marine algae was 5- to 10-fold greater than that for freshwater microalgae (Garzon-Sanabria et al. 2012). Moreover, a commercial product, 'Chitosan', is extensively used for harvesting various microalgae. Chitosan performs flocculation at high efficiency at a low dosage rate, but the efficiency is inhibited in the presence of salt. The optimal dosage of chitosan for flocculation of *Thalassiosira pseudonana* and *Isochrysis* sp. was found to be 40 mg L^{-1} (Harun et al. 2010). However, *Chaetoceros muellari* requires a dosage of 150 mg L^{-1} of chitosan for optimal biomass recovery. The flocculation rate varies with the amount of Chitosan, and its efficiency varies with different algal species (Molina Grima et al. 2003). Flocculation at an elevated pH of 11.8 and 12.0 without any addition of flocculants showed maximum efficiency of 95 %, but the extremely high pH condition can cause adverse effects. Blanchemain and Grizeau (1999) studied recovery of eicosapentaenoic acid (EPA) from *Skeletoma costatum*, where flocculation efficiency of 80 % was found, but the high pH condition of 10.2 caused cell lyses and loss of intracellular contents. An increase in pH condition plays a major role in flocculation. As reported among the five different flocculants (i.e. sodium hydroxide, potassium hydroxide, calcium hydroxide, magnesium hydroxide, and sodium carbonate), flocculation conducted with lime showed the maximum efficiency compared with other base flocculants (Vandamme et al. 2012).

Regardless, the method is easy and very effective for harvesting microalgae culture. However, it is not cost effective and is unsustainable for large-scale harvesting. Chemicals involved in flocculation need to be washed off before it can be re-used, leading to extra operational costs. Further, it can be significantly hazardous to the environment (Salim et al. 2011).

2.4 Flotation

Flotation methods are based on gravity separation in which air or gas is bubbled through a solid-liquid suspension and the gaseous molecules are attached to the solid particles (Chen et al. 2011). The dispersed micro-air bubbles are used to trap the algal cells. In the flotation technique, particle size plays an important role; the smaller the particle, the more likely it is that the particle goes up with the bubbles. The process is dependent on size of the particle, and sizes less than 500 μm can be used during the process (Uduman et al. 2010). Some microalgae also have a natural tendency to float on the water surface due to the high lipid content (Brennan and Owende 2010). The flotation process is divided into three types based on bubble generation: DAF, dispersed flotation, and electrolytic flotation (Chen et al. 2011).

2.4.1 Dissolved Air Flotation (DAF)

The DAF process is the most widely used technique in industries for effluent treatment. The process is based on the reduction in pressure of a water stream that is presaturated with air. The presaturation of the water stream can be carried out with air at pressures higher than atmospheric (Uduman et al. 2010). DAF is often used as an efficient clarification step for treating water containing hydrophobic matter and algae (Barrut et al. 2013). The DAF method involves many factors, such as pressure of the tank, recycle rate, hydraulic retention time, and floating rate of particles. Harvesting of microalgae with DAF combined with flocculation produces 6 % of microalgal slurries. The DAF process coupled with flocculation is usually preferred over settling. The method is effective at large scales, but the usage of chemicals for flocculation can cause adverse effects downstream in the process (Christenson and Sims 2011). A new hybrid system has been studied for simultaneous removal of algae and the organics produced by algae (*Anabaena* and *Mycrocystis*). The system consists of powdered activated carbon (PACs) adsorption and DAF processes, which show low removal efficiency of PACs without coagulation, while efficacy is increased up to 70 % by conventional gravity sedimentation (CGS) and up to 95 % by DAF when coagulant (i.e., polyaluminium chloride) was used (Kwak 2009).

2.4.2 Dispersed Air Flotation

The dispersed air flotation process is based on the formation of bubbles by a high-speed mechanical agitator with an air injection system (Chen et al. 2011). Chen et al. (1998) studied dispersed air flotation using three collectors, i.e. non-ionic X-100, anionic sodium dodecylsulfate (SDS) and N-cetyl-N-N-N-trimethylammonium bromide (CTAB). The recovery of *Scenedesmus quadricauda* was found to be more effective with CTAB (Chen et al. 2011). Effective removal of *Ch. vulgaris* by dispersed air flotation (DiAF) was also studied either using alum in combination with SDS or using dodecylamine alone as the collector (Chen et al. 1998).

The dispersed ozone flotation process is commonly regarded as an expensive process for water and wastewater treatment. It is also used in harvesting microalgae and effluent treatment. Recent study on the recovery of *S. obliquus* FSP-3 with ozonation showed efficient recovery. The ozone-induced flotation leads the algal cells to become more negatively charged and slightly increases the hydrophobicity, favoring the flotation in recovery of *S. obliquus* FSP-3. However, the application of simple air aeration provides hindrance in the formation of flotation (Cheng et al. 2010).

2.4.3 Electroflotation

The electroflotation process involves the application of an electric field to separate the algae. In this method, inactive metal (electrochemically non-depositing) acts as a cathode that generates hydrogen bubbles from water electrolysis. The bubbles produced adhere to the microalgal flocs and carries them to the surface (Chen et al. 2011). The electrolytic flotation method in batch and continuous reactors is studied for microalgae harvesting (Alfafara et al. 2002). The batch study reveals that increasing the input of electrical power increases the removal rate of chlorophyll and decreases the electrolysis time (Uduman et al. 2010). The disadvantages of electrolytic flotation are scaling of the cathode and the high cost. Sandbank and Shelef (1987) investigated the different microalgal recovery techniques and concluded that electroflotation is not the most effective method, but it may be preferred for harvesting marine microalgal species (Uduman et al. 2010). However, a new technique has been introduced for continuous harvesting of microalgae via electro-coagulation-flotation, termed ‘continuous electrolytic microalgae’ (CEM) harvest. This technique generates the polarity exchange with simultaneous harvesting and cultivation of microalgae. During CEM harvest, the polarity exchange creates two distinct phases: phase 1 (P1) and phase 2 (P2). In P1, microalgal cells flocculate due to the destabilization of negatively charged microalgae mediated by metal ions liberated from the dissolving electrode. In P2, the metal ion liberation is terminated and the formation of bubbles from both the electrode causes flocs to float. This technique is efficient in terms of cost but requires further optimization for commercial feasibility (Kim et al. 2012).

2.5 Magnetic Separation

Among the various harvesting processes, magnetic separation has been introduced as a simple technique for efficient recovery of cells and biomolecules from liquid solution with the help of functional magnetic particles driven by an external magnetic field (Wang et al. 2007). Recently, magnetic separation was used in the removal of harmful microalgae by the functionalized magnetic particles. The practical application of this technique is still limited by its complex fabrication and cost (Liu et al. 2009). A recent study on magnetic separation for recovery of *Botryococcus braunii* and *C. ellipsoidea* with naked Fe_3O_4 nanoparticles has been investigated. The recovery efficiency for both of the species was above 98 % within 1 min. The magnetic nanoparticles can be reused and recovered easily from the harvested microalgae biomass, with efficient biomass recovery (Xu et al. 2011). Magnetic harvesting of *C. vulgaris* with iron oxide magnetic particles (IOMMs) prepared by microwave treatment under various conditions (model environment, cultivation media, different pH) showed separation efficiencies of over 95 % at a 3:1 mass ratio of IOMMs to microalgae in presence of phosphorous limitations. The interactions of algae–IOMMs are essential for magnetic cell separations, and phosphorus ions have been identified as an interfering element in the culture medium responsible for microalgae harvesting (Prochazkova et al. 2013). Magnetophoresis has also been applied in the separation of microalgae blooms in commercial fish production ponds. A study conducted using iron oxide magnetic nanoparticles (IONPs) for microalgae separation in the aquaculture industry showed recovery efficiency of 90 % in less than 3 min (Toh et al. 2012). This magnetic separation technology provides efficient microalgae recovery with less energy input and time.

Table 1 Comparison of different microalgae harvesting methods

Methods	Recovery	Scale	Energy consumption (kWh/m^3)	Benefits	Limitations
Centrifugation	90 %	Bench	8	Reliable, high solid concentrate	Energy intensive, expensive
Flocculation	80–95 %	Pilot	14.81–0.33	Good recovery	Flocculants can be expensive, may cause contamination issues
Tangential flow filtration	70–89 %	Bench	0.38–2.06	Reliable, high solid concentrate	Membrane fouling, high cost
Dissolved air flotation	80–90 %	Pilot	7.6	Proven in large scale	Use of flocculants may create problems
Electroflotation	>95 %	Bench	–	Efficient recovery	High cost, electrodes need to be replaced periodically
Forward osmosis	80–92 %	Pilot	0.3	Low energy input	Slow and problems in reverse solute flux
Magnetic separation	90–98 %	Bench	–	Rapid, less energy intensive, and environmental friendly	Complex fabrication and expensive

Adapted from Christenson and Sims (2011), Xu et al. (2011), and Buckwalter et al. (2013)

The recovery, energy consumption, and advantages of different types of harvesting methods is shown in Table 1.

3 Selection of Harvesting Method

Selection of able harvesting technology is a crucial step for further downstream processing and economic production of microalgal biomass (Brennan and Owende 2010). The method must be applicable to the harvesting of large volumes of microalgae culture and must take into consideration the acceptable amount of moisture content in the product (Molina Grima et al. 2003). The feasible product formation also intended to choose a suitable technology for harvesting microalgae biomass. The harvesting technology is dependent on microalgae characteristics such as size and density (Olaizola 2003). Selection of strain must be taken into account for the processing method, as the ease of harvesting varies between species. For example, gravity settling and conventional filtration processes are suitable for large-sized microalgal cells (>70 mm) such as *Coelastrum* and *Spirulina* sp. (Brennan and Owende 2010; Buckwalter et al. 2013). On the other hand, membrane filtration and ultra-filtration are technically feasible for small-sized algal cells (<30 mm) (Brennan and Owende 2010).

4 Harvesting Techniques in Industry

The high commercial demand for microalgae for wastewater treatment, production of biofuels, and other value-added products have brought forward the research and development of various microalgae by private companies and industries (Christenson and Sims 2011). Large-scale biomass production under various cultivation systems highlights the challenges of harvesting techniques for further downstream processing. The mass production of marine microalgae cyanobacteria *Spirulina* sp. has long been used for various neutraceutical products. Companies like Earthrise Nutritionals and Cyanotech are involved in cultivating *Spirulina* sp. in open raceway ponds in clean and non-wastewater systems (Christenson and Sims 2011). Because of the filamentous structure, simple filtrations methods for harvesting *Spirulina* were very effective. A patent application by Solix Biofuels and A2BE

Carbon Capture described a closed reactor system with a rotatable internal transparent insulator. The reactor is designed with a harvesting chamber where fluid motion maintains a whirlpool to pre-concentrate the algae before it is passed through a roller press (Sears 2007). Algae to Energy (or A2E) describe a harvester named the ‘Shepherd harvester’, which consists of a continuous belt and a vacuum system that moves through the algae culture. While moving, any algae collected on the belt are harvested by the vacuum system before the belt passes through the culture again (Christenson and Sims 2011). Another patent application by Algaeventure Systems Inc. also describes the use of a continuous belt harvester. The harvester is based on capillary extraction and consists of a primary and a secondary belt (Youngs and Cook 2010). The function of the primary belt is to collect algae, and the secondary belt attached to the bottom portion of the primary belt is compressed to drain water after the dried biomass on the primary belt is collected. Another two energy companies, MBD Energy and Scipio Biofuels, also use centrifugation methods to harvest microalgae. MBD Energy is in collaboration with a Dutch company, Evados, and uses their separators. The Evados separator is a centrifuge; it allows for easier removal of solids after concentration. However, Scipio Biofuels also uses a centrifugation process but of a low speed for harvesting (Christenson and Sims 2011). Sapphire Energy genetically modified the algae to enable controlled flocculation and simple harvesting. Another patent application by Kent Bioenergy describes a series of decanting procedures to initiate the culture for flocculation. In this method, the flow of culture in a raceway pond is blocked, and the upper layer of the water with the culture is removed, and an equal amount of removed culture volume is replenished. The process is repeated a couple of times until the sediment-ready algae sufficiently dominate the culture (Schwartz et al. 2010). In terms of biological-based harvesting, Algenol Biofuels Inc. tried genetic modification of microalgae to avoid the biomass harvesting step (Woods et al. 2010).

5 Possible Alternatives for Economic and Environmental Friendly Harvesting

Recovery of microalgae biomass on a commercial scale may be possible using different harvesting methods, i.e. centrifugation, filtration, and flocculation. However, these methods also have many disadvantages like investment of high capital, energy, and running costs and are considered mostly unlikely to be economical on a commercial scale (Becker 1994; Benemann and Oswald 1996; Lee et al. 2009). For commercial production of microalgal products, the harvesting technique must consider the harvesting cost, energy input, and environmental effect.

5.1 Bioflocculation

Bio-based flocculation has emerged as a new alternative for eco-friendly harvesting of algal biomass. Generally, bioflocculation is a dynamic process derived from extracellular polymer substances (EPS) synthesized by living cells. Microorganisms such as bacteria, fungi, and actinomycetes have been known to produce EPS such as polysaccharides, functional proteins, and glycoprotein, which act as bioflocculant (Lam and Lee 2012). Bioflocculation with bacteria have been also applied in wastewater treatment. However, these processes require additional substrates for bacterial growth, and bacterial contaminations are promoted, which create a hindrance to the recovery of the algal biomass (Salim et al. 2011). Apart from this, bioflocculation induced with environmental stresses like high temperature and pH or nutrient depletion may affect cell composition, and the process is considered uneconomical on a commercial scale (Lee et al. 2009). An assessment was carried out to estimate the cost and energy consumption for utilizing bioflocculation on a commercial scale. In the assessment, the microalgae harvesting system is incorporated with a baffled hydraulic flocculator for mixing the process, and the overall energy consumed per ton of flocculated dry biomass was estimated to be 0.9 kWh or the equivalent of 9.8 MJ tone^{-1} biodiesel (assuming microalgae lipid content is 33 %). The cost of this harvesting process was estimated to be 79 % lower than other conventional flocculants (Lee et al. 2010). The harvested culture medium can be effectively reused for microalgae cultivation without affecting its growth, and thereby the process reduces the other additional costs such as those for water treatment and purification (Lam and Lee 2012). Another study with microbial flocculation for harvesting marine microalgae was conducted by Lee et al. 2010. The growth of flocculating microbes was enhanced by supplementing organic carbon (acetate, glucose, or glycerin) as a substrate. The EPSs were induced by providing nutrient stress, and an average recovery efficiency of 90 % was achieved at a low concentration of organic substrate (0.1 g L^{-1}) (Lam and Lee 2012).

5.2 Immobilization Biotechnology

The growing harvesting challenges have developed an interest in using immobilization techniques for harvesting. The method is based on entrapment of microalgae in a matrix and it allows continuous growth of the cells within the matrix. When the microalgae reach a stationary growth phase, the immobilized microalgae cells will settle at the bottom of the culture medium. In this case, the energy requirement may not be high, as the relatively large microalgae beads can be harvested with a simple filtration method (e.g., sieve) (Christenson and Sims 2011). Various immobilization techniques have been developed and, so far, entrapment with alginate gel is the most feasible for immobilization of microalgae. The alginate gel entrapment method has some advantages like negligible toxicity, high transparency, and a requirement for mild conditions during the immobilization process (Moreno-Garrido 2008). The incorporation of the immobilization technique in biofuel production is believed to play a successful role in the commercial scale (Lam and Lee 2012). However, the immobilization of microalgae is also associated with some disadvantages, such as (1) dissolution of beads due to the high presence of anti-gelling cations in sea water and serious microbial attack causing loss of microalgae cells (Mallick 2006), (2) rupture of beads, which is caused by insufficient divalent ions (Lam and Lee 2012), (3) mass transfer limitation due to the formation of bio-film as a protection layer to the microalgae cells, hindering the transport of nutrients and carbon source. Hence, the immobilized microalgae show much slow growth compared with free cell culture, and the problem can be solved by co-immobilization with plant growth-promoting bacteria (PGPB) (Gonzalez and Bashan 2000).

6 Conclusions

In the literature, several methods for the harvesting of microalgae biomass have been studied, and many challenges associated with the method have also come to light. The varied morphology of microalgal species and the final desired product have led to the recovery of biomass becoming more challenging in itself. In addition, the economical and environmental aspects, and the energy requirements, of microalgae harvesting on a commercial scale must be investigated. Centrifugation is the most efficient of the discussed harvesting methods, but it has some drawbacks due to the requirement for a high level of energy and the associated costs. Many companies are undertaking various research and development in the harvesting of microalgae biomass. However, microalgae harvesting on a large scale still needs considerable design and technological development to reduce the cost of the method and become more commercially viable.

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Part III

Remediation Technologies

Characterization of *Bacillus* Strains Producing Biosurfactants

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Abstract

Genus *Bacillus* includes species of industrial, biotechnological, and environmental interest, as well as clinically important strains. In terms of metabolic properties, they present a diverse group, as they can degrade various substrates and produce many molecules, including lipopeptide (LP) biosurfactants. Due to a high interest in biosurfactants for application in different fields, the molecular mechanisms of regulation of the expression of the operons responsible for LPs have been intensively studied. Additionally, many assays have been created to evaluate the use of cost-effective renewable agro-industrial substrates for production. The purpose of the chapter is to provide a comprehensive overview of the results of our studies on identification, characterization, and assessment ability of three *Bacillus* strains to produce biosurfactants and detection of genes encoding enzymes involved in biosurfactant synthesis. Moreover, the use of alternative substrates to decrease the cost of LP biosurfactant production and some aspects of application of *Bacillus* spp. as biocontrol agents are discussed.

Keywords

Bacillus strains • Biosurfactants • Mesophiles • Thermophiles

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1 Introduction

Genus *Bacillus* encompasses a Gram-positive, endospore-forming, rod-shaped bacteria, obligate aerobes, or facultative anaerobes. The bacilli includes species of industrial, biotechnological, and environmental interest, as well as clinically important strains. Members of genus *Bacillus* are widely distributed in the environment and have ecological importance. Since this group encompasses closely related strains, their identification can be difficult. Therefore, the use of a combination of biochemical, metabolic, and molecular methods is required for accurate discrimination. In terms of metabolic properties, they present a diverse group, as they can degrade various substrates and produce many molecules, including lipopeptide (LP) biosurfactants, compounds composed of cyclic peptides linked to various fatty acids. Since this group of biosurfactants exhibits, besides the surface active properties, antibacterial, antifungal, antiviral, and antitumor activities, they are commonly used in agriculture, food production, chemistry, cosmetics, pharmaceutics, and environmental biotechnology. Biosurfactants have numerous beneficial qualities: they can be non-toxic, non-hazardous, biodegradable, environmentally friendly, selective, and effective under extreme conditions, and have numerous industrial applications and unique surface-active properties. In spite of these beneficial properties, their higher production cost compared with synthetic surfactants is a major drawback. Biosurfactants could potentially replace synthetic surfactants if the cost of their production were to be substantially lowered. The best way to reduce substrate costs for biotechnology at present is to use recycled agricultural material with the right balance of nutrients to support microbial growth and biosurfactant production for environmental applications. So far, several renewable substrates, including various agricultural and industrial by-products and waste materials, have been intensively studied for microorganism cultivation and biosurfactant production at the laboratory scale. These include olive oil mill effluent, waste frying oil, oil refinery wastes, soapstock, molasses, whey, starch wastes, cassava-flour processing effluent, and distillery waste (Makkar and Cameotra 2002; Deleu and Paquot 2007). Value-added products or benefits can improve the economics of such bioprocesses, including microbial waste reduction.

Due to the high interest in biosurfactants for application in a variety of fields, the molecular mechanisms of regulation of the expression of the operons responsible for LPs as well as identification of genes responsible for their synthesis have been studied (Jacques 2011). The purpose of the chapter is to provide a comprehensive overview of the results from our studies on identification, characterization, and assessment ability of three *Bacillus* strains to produce biosurfactants, and detection of genes encoding enzymes involved in biosurfactant synthesis. Moreover, the use of alternative substrates to decrease the cost of LP biosurfactant production and some aspects of *Bacillus* spp. application as biocontrol agents are also discussed.

2 Genus *Bacillus*

Genus *Bacillus* encompasses a gram-positive, endospore-forming, rod-shaped bacteria, obligate aerobes, or facultative anaerobes. The first classifications of *Bacillus* species were based on two characteristics: aerobic growth and endospore formation. This resulted in linking together many bacteria possessing different kinds of physiology, ecology, and genetics, making it difficult to categorize the genus *Bacillus* or to make generalizations about it. Subsequently, numerical classification based on a series of phenetic characters has been used for the classification of 368 *Bacillus* strains into 79 clusters (Priest et al. 1988). At about the same time, ribosomal DNA (rDNA) sequences were being established as a very useful molecular marker to infer phylogenetic relationships and, after 1990, 16S rDNA sequence alignment has been successfully applied in determining phylogenetic relationships of *Bacillus* species. Now, based on the genetic approach, genus *Bacillus* belongs to the division *Firmicutes*, order *Bacillales*, and the family

Bacilliaceae. Various studies have been conducted on the genetic diversity of the genus *Bacillus*. Using 16S rDNA sequence analysis, Ash et al. (1991) distinguished five phylogenetic groups in this genus. In turn, Nielsen et al. (1994) described a sixth belonging to the alkaliphilic bacilli. Then, Xu and Côté (2003), using both 16S rDNA and 16S-23S ITS nucleotide sequences, analysed the phylogenetic relationship between bacilli species and divided them into ten distinct clusters.

Since the genus *Bacillus* includes species that are of industrial, biotechnological, and environmental interest, as well as clinically important species, their biochemical characteristics have been intensively studied. They are widely distributed in the environment and tolerate various environmental conditions. The majority is mesophiles, with an optimal temperature of 30–45 °C; however, some are thermophiles, growing at even 65 °C, or psychrophiles, able to grow at 0 °C. They are found growing over a range of pH from 2 to 11. In general, bacilli are capable of using simple organic compounds, such as amino acids, sugars, organic acids, or carbohydrates, as well as various other substances.

The *Bacillus* strains named T-1, T′-1, and I′-1a were isolated from 100-year-old oil refinery sludge in Czechowice-Dziedzice (Poland) as described by Berry et al. (2006). The morphological and biochemical properties of these strains are listed in Table 1.

Table 1 Biochemical characteristics of *Bacillus* strains isolated from oil refinery waste

	Strains		
Test	T-1	T′-1	I′-1a
Gram-staining	+	+	+
Growth temperature:			
30 °C	+	+	+
37 °C	+	+	+
45 °C	+	+	+
65 °C	–	–	–
Sporulation	+	+	+
Salinity (% NaCl):			
2	+	+	+
4	+	+	+
8	+	+	+
12	+	–	+
Utilization of sodium acetate	+	+	+
Tween 20	+	+	+
Tween 80	+	+	+
Indol production	+	+	+
Glucose (acidification)	+	+	+
Arginine (arginine dihydrolase)	–	–	–
Urea (urease)	–	–	–
Esculin (hydrolysis β -glucosidase)	+	+	+
Gelatine (hydrolysis)	+	+	+
Glucose (assimilation)	+	+	+
Arabinose (assimilation)	+	+	+
Mannowe (assimilation)	+	+	+
Mannitol (assimilation)	+	+	+
N-acetyl-glucosamine (assimilation)	+	+	+
Maltose (assimilation)	+	+	+
Gluconate (assimilation)	+	+	+
Caprate (assimilation)	–	–	–
Adipate (assimilation)	–	+	–
Malaje (assimilation)	+	+	+
Citrate (assimilation)	+	+	+
Alcaline phosphatase	+	+	+
Esterase (C4)	+	+	+
Esterase lipase (C8)	+	+	+
Lipase (C14)	–	–	+
Acid phosphatase	+	+	+

According to Płaza et al. (2010)
\+ positive reaction; – negative reaction

3 Identification of *Bacillus* Strains

Taxonomic analysis of species of genus *Bacillus* carries some difficulties (Guinebretiere et al. 2001; De Paolis and Lippi 2008). They constitute closely related taxa, and their differentiation and identification using one method is often impossible and requires the combination of biochemical, metabolic, and molecular methods (Ash et al. 1993; Chun and Bae 2000; Guinebretiere et al. 2001; Suihko et al. 2004; Hutsebaut et al. 2006; De Paolis and Lippi 2008; Płaza et al. 2012). In our study, three methods were used to identify *Bacillus* strains: 16S rDNA sequencing, the Biolog system, and fatty acid analysis.

Only one isolate (I′-1a) was successfully identified as *B. subtilis* by the 16S rDNA gene sequence analysis, while T-1 and T′-1 were

Table 2 Identification of *Bacillus* strains

	Bacteria identification methods		
Bacillus species	Biolog system	16S rRNA	FAME
T-1	*B. subtilis/atrophaeus*	*B. subtilis & B. licheniformis*	*B. subtilis*
T′-1	*B. subtilis ss spizizenii*	*B. subtilis & B. amyloliquefaciens*	*B. amyloliquefaciens*
I′-1a	*B. licheniformis*	*B. subtilis*	*B. subtilis*

identified as *Bacillus* genus. 16S rDNA gene sequencing could not clearly assign them to any species of *Bacillus*, as both isolates showed >99 % similarity to two distinct species (*B. subtilis* and *B. licheniformis* for T-1 and *B. subtilis* and *B. amyloliquefaciens* for T′-1).

The analysis of 16S rDNA sequences is powerful tool for the classification of micro-organisms; however, the presence of highly conserved sequences in this gene sometimes disenables the distinction among closely related *Bacillus* species and sub-species (Guinebretiere et al. 2001; Cho et al. 2004; Wu et al. 2006; Miranda et al. 2008). Another taxonomic marker used for identification of micro-organisms was fatty acid methyl esters (FAMEs). The application of FAME analysis for differentiation of *Bacillus* strains and identified by the 16S rDNA method did not bring a clear differentiation between T-1 and I′-1a strains (Table 2). This is because the genus *Bacillus* consists of the *Bacillus cereus* and the *Bacillus subtilis* groups, containing the species with almost indistinguishable fatty acid patterns, and the studied strains belonged to one of them.

The last method used to clarify an ambiguous 16S rDNA and FAME identification of three *Bacillus* isolates was the Biolog system. The metabolic profile of 94 biochemical tests as measured by the BIOLOG™ system, showed identification matches for all three *Bacillus* spp. The T-1 strain was assigned to the species *B. subtilis* or *B. atrophaeus* with a SIM value of 0.593 and 0.549, respectively, based on its utilization pattern of 37 substrates. The two strains T′-1 and I′-1a were identified as *B. subtilis ss spizizenii*, with a SIM value of 0.567 and utilizing 68 substrates, while *B. licheniformis*, with a SIM value of 0.562 utilized 50 substrates, respectively (Table 2). Nevertheless, the results obtained did not permit the unequivocal identification of the analysed strain and confirmed that bacilli are a closely related group and are considered difficult to identify with metabolic profiles, as reported by other authors (Baillie et al. 1995; Guinebretiere et al. 2001).

As shown above, the 16S rDNA gene sequence, and metabolic and fatty acid profile analyses are often insufficient for accurate discrimination between *Bacillus* strains. Therefore, Chun and Bae (2000) proposed the use of a partial gyrA sequence, coding for DNA gyrase subunit A, as a useful marker for the determination of phylogenetic relationships. Moreover, it allows accurate classification of *B. subtilis* and related taxa, including *B. licheniformis*, *B. mojavensis*, *B. amyloliquefaciens*, and *B. atrophaeus*. Abella et al. (2000) verified the feasibility of using fluorescein-labeled trinucleotides as "quantitative probes" for the identification of bacilli species. Labeled TTT, GGG, and ATA triplets in 16S rRNA sequences were hybridized to the ribonucleic acid of *Bacillus* spp. whole cells and the number of the triplets was quantified by synchronous fluorescence spectrometry.

A new technique currently being studied for identification of bacilli strains is Raman spectroscopy. This technique provides complex information about chemical cell components, such as carbohydrates, fatty acids, proteins, and nucleic acids, and hence combines the discriminatory abilities of various phenotypic characteristics (Hutsebaut et al. 2006). As shown, Raman spectroscopy enabled the unambiguous identification of at least 90 % of tested strains.

4 Production of Biosurfactants by *Bacillus* Strains

Many strains from genus *Bacillus* have the ability to produce biological surface active compounds (biosurfactants) belonging to the LP group. This group is classified into four main families: surfactins, iturins, fengycins (or plipastatins), and (discovered in the year 2000) kurstakins. Each family of LPs is composed of several compounds, among which the most common are surfactin, lichenysin A, pumilacidin, and bamylocin A for the family of surfactins; iturin A and C, bacillomycin D, F, L, and Lc and mycosubtilin for iturins; and fengycin A and B for the fengycin family (Jacques 2011). In general, LPs consist of cyclic peptides composed of seven (surfactins and iturins) or ten (fengycins) α-amino acids linked to one unique β-amino (iturins) or β-hydroxy (surfactins and fengycins) fatty acid. The length of this fatty acid chain may vary from C_{13} to C_{16} for surfactins, from C_{14} to C_{17} for iturins, and from C_{14} to C_{18} for fengycins. Different homologous compounds for each LP family are usually co-produced (Akpa et al. 2001).

Because the LP biosurfactants exhibit a great ability to reduce surface and interfacial tension, they are excellent emulsifiers, foaming, and dispersing agents widely used in many industries such as agriculture, food production, chemistry, cosmetics, pharmaceutics, and environmental biotechnology (Pacwa-Płociniczak et al. 2011). LPs, similar to other biosurfactants, are environmentally friendly, biodegradable, low toxic, and non-hazardous. They have better foaming properties and higher selectivity. They are active at extreme pH and salinity conditions, but, most importantly, they work efficiently at high temperatures. For example, thermotolerant biosurfactant-producing strains of *Bacillus* able to grow at temperatures up to 50 °C or even 73 °C have been successfully used in microbial-enhanced oil recovery and oil-sludge clean-up (Banat 1993; Jinfeng et al. 2005).

5 Methods for Detection of Biosurfactant-Producing Bacillus Strains

In general, methods used for assessment of the bacterial biosurfactant production abilities can be divided into indirect (involving methods of cultivation of bacteria on specific media, analysis of surface activity of tested strains or their emulsifying ability, or cell surface hydrophobicity measurement) and direct (based on the measurement of surface/interfacial tension of a medium after bacterial cultivation) (Fig. 1). A new approach for the determination of the potential ability of *Bacillus* strains for biosurfactant production is the detection of genes encoding enzymes involved in LP biosynthesis (Satpute et al. 2010; Walter et al. 2010).

In our studies, measurement of the surface tension, drop-collapse, oil-spreading, and blood-agar tests were used to determine the ability of bacilli for production of LPs when growing on the chosen agro-industrial wastes (Table 3). Additionally, to confirm the presence of genes encoding enzymes involved in LP synthesis in T-1, T′-1, and I′-1a *Bacillus* strains, the polymerase chain reaction with primer pairs proposed by Tapi et al. (2010) and Hsieh et al. (2004) were used (Fig. 1).

The obtained results showed that strain T′-1 had the potential to produce three types of LPs, i.e. fengycin, mycosubtilin/iturin, and surfactin/lichenysin. However, this approach did not make it possible to differentiate between mycosubtilin and iturin, as well as surfactin and lichenysin. In the study with the primer for the sfp gene, the ability to produce surfactin was confirmed only for strain T-1. The use of primers for surfactin/lichenysin proposed by Tapi et al. (2010) allowed us to obtain a product for T′-1 and I′-1a strains (Fig. 2). This observation may be because, in these two strains, surfactin is encoded by srfA gene or they produce lichenysin only.

Mass spectrometry with electrospray ionization and nuclear magnetic resonance (NMR) (400 MHz) were also used to characterize the purified surfactants. The preliminary chemical analysis showed the LPs constituted rich fractions of supernatants obtained from the cultures of three *Bacillus* growing on molasses and brewery effluents. The characteristic m/z peaks of surfactin, fengycin, and iturin families were observed in the analysed samples. Also, a novel unknown product was isolated from *Bacillus* named T′-1 growing on molasses. Its chemical structure is under evaluation by Poliwoda et al. (2012).

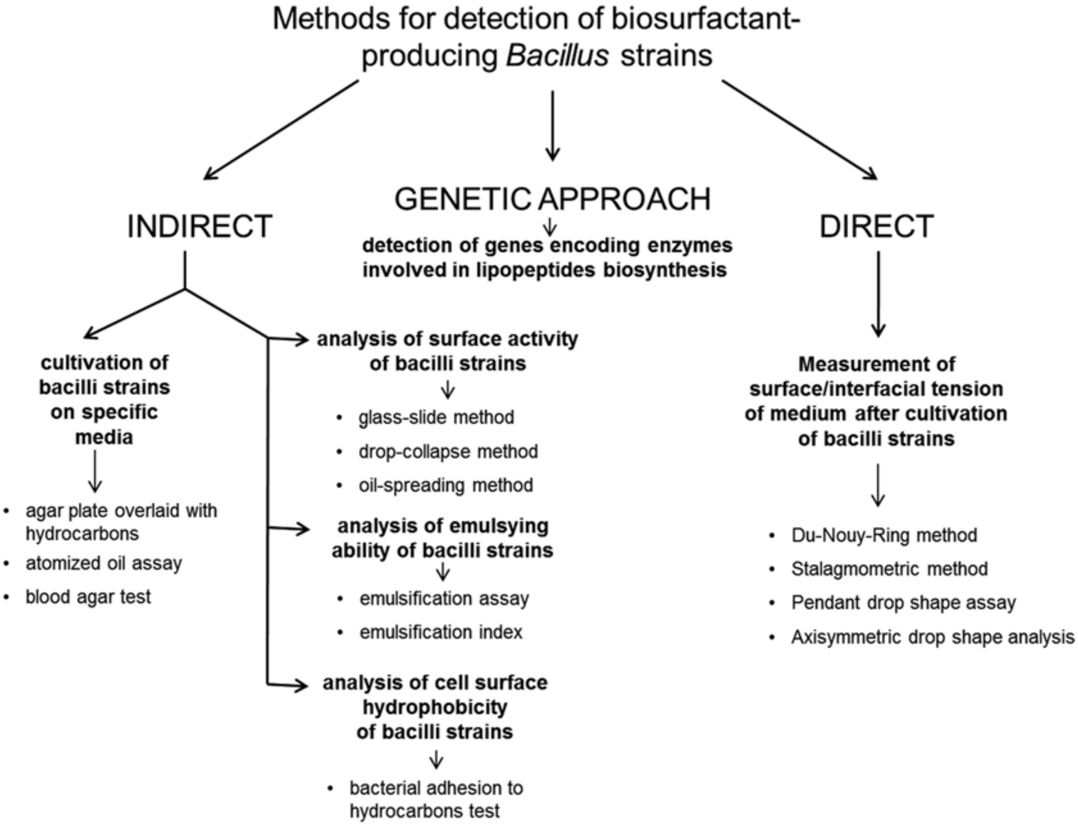

Fig. 1 Methods used for detection of biosurfactant-producing *Bacillus* strains

Table 3 Characterization of surface active properties of isolates grown in liquid media composed of different waste products

Waste products	Strains	Surface tension (mN/m)	Drop-collapse method	Oil spread method (mm)	Hemolytic activity
Brewery effluent 1	T-1	37.73 ± 1.05	+	4.67	+
	T′-1	42.73 ± 0.58	−	17.67	+
	I′-1a	33.05 ± 0.17	+	6	+
Brewery effluent 3	T-1	34.43 ± 0.30	+	4.33	+
	T′-1	39.21 ± 0.98	−	4	+
	I′-1a	31.54 ± 0.57	+	10	+
Molasses	T-1	26.64 ± 0.43	+	28.33	+
	T′-1	29.25 ± 0.93	+	40	+
	I′-1a	28.45 ± 0.54	+	40	+
Fruit and vegetable decoction	T-1	31.55 ± 0.91	−	2.33	+
	T′-1	36.55 ± 0.28	−	2.67	+
	I′-1a	30.82 ± 0.64	−	6.67	+

According to Płaza et al. (2011)

Surface tension (ST) data are means ± standard deviation of three independent experiments; ST of water: 71.79 ± 0.3 mN/m

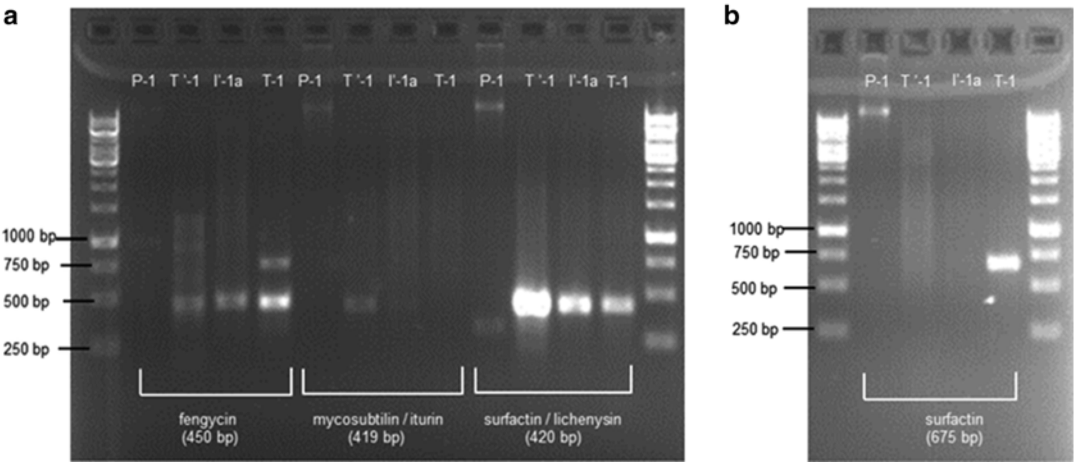

Fig. 2 Agarose gel electrophoresis of polymerase chain reaction products of genes encoding lipopeptides in *Bacillus* strains (T′-1, I′-1a, and T-1) and *Pseudomonas* sp. (P-1, control). (**a**) – primer pairs: Af2-F/Tf1-R (fengycin), Am1-F/Tm1-R (mycosubtilin/iturin), and As1-F/Ts2-R (surfactin/lichenysin), (**b**) – primer pair sfp (surfactin)

So far, one of the most often studied genes for the screening of the ability of *Bacillus* strains to surfactin production has been sfp (Hsieh et al. 2004; Hassan et al. 2010). The new approach for the detection of non-ribosomal peptide synthetases (NRPSs) and hybrid polyketide synthetases (PKS-NRPSs) genes coding enzymes involved in synthesis of LPs was proposed by Tapi et al. (2010). They designed four primer pairs, named As1-F/Ts2-R, Am1-F/Tm1-R, Ap1-F/Tp1-R, and Af2-F/Tf1-R. The proposed approach allows the detection of the presence of both NRPSs and PKS-NRPSs genes in analysed bacilli (Tapi et al. 2010). Apart from genes responsible for biosurfactant production, the mechanism of regulation of the expression of the operon involved in biosynthesis has been intensively studied (Jacques 2011). It has been found that production of LPs is a cell-density-responsive mechanism utilizing several pheromones, ComX, PhrC, PhrG, and PhrH, and transcription factors, such as CodY, DegU, and AbrB. LPs of *Bacillus subtilis* are synthesized by NRPSs or by PKS-NRPSs (Ongena and Jacques 2008). They are known as enzymes involved in the biosynthesis of several hundred bioactive compounds. These synthetases consist of functional units called modules that catalyze the different reactions necessary in peptide biosynthesis. Each module is sub-divided into several catalytic domains responsible for each biochemical step. A typical NRPSs module comprises about 1,000 amino acid residues and is responsible for one reaction cycle of selective substrate recognition and activation (Ongena and Jacques 2008; Tapi et al. 2010). The operons of the surfactin family contained four open reading frames (ORFs) coding for surfactin end licheny-sis synthetases, which are designed srfA-A, srfA-B, srfA-C, and srfA-D or lchA, lchB, lchC, and lchD (Tapi et al. 2010). Similarly, plipastatin or fengycin are synthesized by NRPSs by an operon with five ORFs, namely ppsA, ppsB, ppsC, ppsD, and ppsE (fenC, fend, fenE, and fenB). In turn, iturin molecules are synthesized by PKS multi-modular enzymes encoded by an operon that consists of four ORFs called fenE, mycA, mycB, mycC, or ituD, ituA, ituB, and ituC for mycosubtilin or iturin, respectively.

Apart from genes responsible for biosurfactant production, the mechanism of regulation of the expression of the operon involved in biosynthesis is as yet completely unknown. Most have focused on the regulation of surfactin. Production of this biosurfactant is a cell-density-responsive mechanism not based on homoserine lactone but utilizing several pheromones, ComX, PhrC, PhrG, and PhrH and transcription factors, such as CodY, DegU, and AbrB. Accumulation of these pheromones in the growth medium causes expression of the srfA operon. Two types of pheromones are involved in the regulation in this operon. The first pheromone is ComX, which is secreted outside the cell; when its concentration is critical, ComP senses it and phosphorylates itself. Phosphorylation of ComA by phosphorylated ComP is then observed. It activates expression of surfactin operon and ComS, which is an ORF encoded within the srfAmRNA. In addition, several activators (e.g. DegU) and repressors (e.g. CodY and AbrB) are involved in ComK activation. srf transcription is also activated by a second pheromone CSF by inhibiting the ComA-phosphate phosphatise RapC (Jacques 2011).

6 Unconventional Media for Production of Biosurfactants by *Bacillus* Strains

Interest in biosurfactant applications in many industries and environmental protection has significantly increased recently. However, their large-scale application is limited due to the high cost of production. Therefore, many efforts have been made to reduce these costs. The success of biosurfactant production depends on the increase of yield, development of economical engineering processes, and the use of low-cost effective renewable agro-industrial substrates for their production. The search for inexpensive raw materials is important to the overall economy of biosurfactant production since they account for 10–30 % of the total cost (Cameotra and Makkar 1998; Makkar et al. 2011). The main problem in the use of raw substrates is selection of suitable waste materials with the right balance of nutrients sufficient for cell growth and biosurfactant accumulation.

In our research, the potential of agro-industrial wastes to replace synthetic media to support growth of the *Bacillus* strains and biosurfactant synthesis was investigated (Płaza et al. 2010, 2011). The growth of bacteria was evaluated in solid and liquid media. The results of growing *Bacillus* strains on solid media are presented in Table 4.

Table 4 Growth of *Bacillus* strains on solid media composed of different waste products

	Bacillus strains		
Waste Products	T-1	T′-1	I′-1a
1. Whey 1	–	–	–
2. Dairy wastewater	–	++	+
3. Whey 2	–	–	–
4. Brewery effluent 1	++	+++	+++
5. Brewery effluent 2	+	+	+
6. Brewery effluent 3	+++	+++	+++
7. Brewery spent grain	++	+	+
8. Sugar wastewaters	++	+	–
9. Beet pulp	+++	++++	+++
10. Molasses	++++	++++	++++
11. Soapstock 1	–	–	–
12. Soapstock 2	–	–	–
13. Oil slime	–	–	–
14. Acidic fatty wastewater 1	–	–	–
15. Acidic fatty wastewater 2	–	–	–
16. Fusel	–	–	–
17. Slop*	+++	+++	+++
18. Potato decoction	++	++	++
19. Apple pomace	+	+	+
20. Citrus pomace	+	+	+
21. Fruit and vegetable decoction	+++	+++	+++

According to Płaza et al. (2011)
Concentration of wastes – 100 % (v/v); * concentration of slop – 50 %; ++++ very good growth; +++ good growth, ++ middle growth, + poor growth, – no growth

Bacillus strains grew very well on solid media containing two brewery effluents, beet pulp, molasses, slop, and a fruit and vegetable decoction. These substrates appeared to be a good candidate to replace the conventional media, probably due to the right balance of carbohydrates and lipids to support optimal growth of the strains. Brewery effluents, molasses, and the fruit and vegetable decoction were then tested as substrates for growth of the isolates in liquid media. Among them, molasses, the fruit and vegetable decoction, and brewery effluents were found to be the best alternative media for growth of tested *Bacillus* strains. All substrates had high values of chemical oxygen demand (COD) and biochemical oxygen demand (BOD), and nutritional components of the wastes were efficiently utilized for biomass build-up. Finally, determination of biosurfactant properties of isolates grown in liquid media composed of different waste products showed that the best unconventional substrates for bacteria growth and biosurfactant production were molasses and brewery effluents.

7 *Bacillus* Strains as Biocontrol Agents

Biological control has emerged as a promising alternative to chemical pesticides. Currently, the use of beneficial micro-organisms (biopesticides) is considered the most promising method for environmentally friendly crop-management practices. Members of the *Bacillus* genus produce a variety of biologically active molecules that have been identified as microbial pesticides, fungicides, or fertilizers (Ongena and Jacques 2008; Pérez-Garcia et al. 2011). Among these compounds, cyclic LPs of the surfactin, iturin, and fengycin families have well recognized potential for use in plant disease biocontrol. In our study, the antifungal activity of *Bacillus* strains growing on agro-industrial wastes and their cell-free supernatants as a source of antifungal agents and biosurfactants was evaluated. Mycelium growth inhibition by active *Bacillus* broth cultures was evident (Płaza et al. 2012). The results demonstrated the ability of the *Bacillus* strains grown on brewery effluents and molasses to inhibit the mycelial growth of tested phytopathogenic fungi (Fig. 3a–c). Great mycelial inhibition was observed for the following fungi: *B. cinerea* A 258, *Phomopsis viticola* W 977, *Septoria carvi* K 2082, *Colletotrichum gloeosporioides* A 259, *Phoma complanata* A 233, and *Phoma exigua var. exigua* A 175. Inhibition of the mycelial growth of fungal species by the cell-free supernatants as sources of antifungal agents was also observed (Fig. 3d–f). Fungi appeared more sensitive

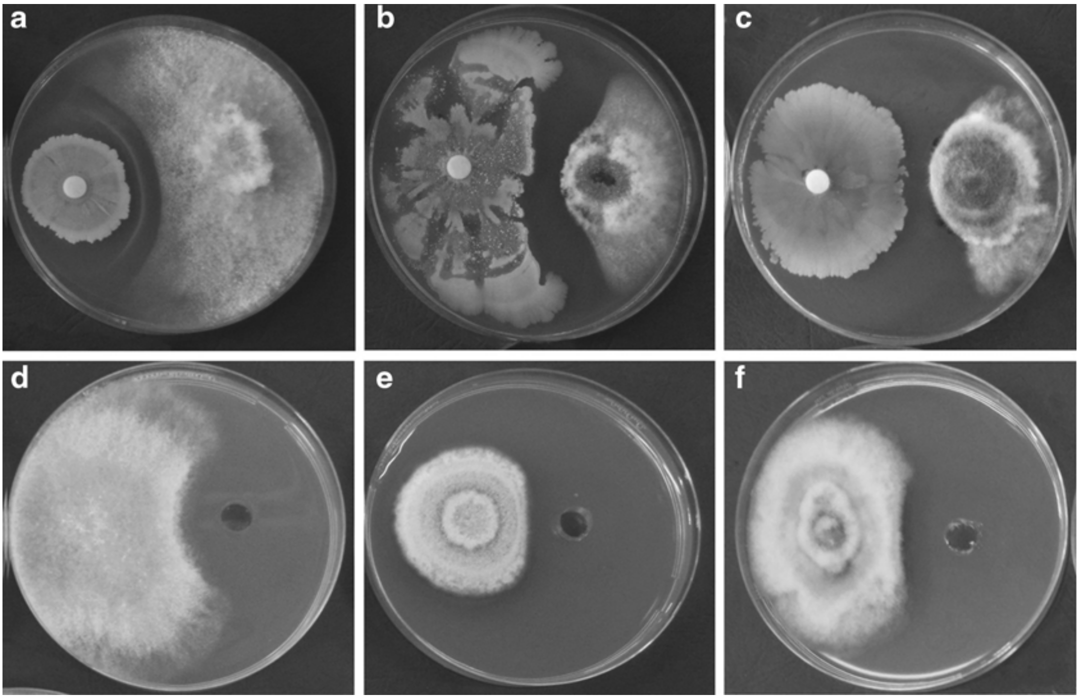

Fig. 3 Mycelium growth inhibition by *Bacillus* strains (**a**–**c**) and cell-free supernatants (**d**–**f**); (**a**) – inhibition of *Botrytis cinerea* A 258 by I′-1a strain grown on molasses; (**b**) – inhibition of *Colletotrichum gloeosporioides* A 259 by I′-1a strain grown on brewery effluents (# 6); (**c**) – inhibition of *Phoma exigua var. exigua* A 175 by T′-1 strain grown on brewery effluents (# 4); (**d**) – inhibition of *Botrytis cinerea* A 258 by T′-1 strain grown on molasses; (**e**) – inhibition of *Colletotrichum dematium* K 425 by I′-1a strain grown on molasses; (**f**) – inhibition of *Phoma exigua var. exigua* A 175 by T-1 strain grown on molasses

towards supernatants obtained from the molasses broth cultures. The inhibition was observed for *B. cinerea* A 258, *R. solani* W 70, *S. sclerotiorum* K 2291, *Phomopsis diachenii* K 657, *C. dematium* K 425, *P. complanata* A 233, and *P. exigua var. exigua* A 175. However, the supernatants obtained from the brewery cultures inhibited mycelium growth of *Colletotrichum* dematium K 425 and *Phoma* species. The results obtained may be a result of variable production of metabolites by *Bacillus* strains because Gordillo et al. (2009) demonstrated that culture media composition may also influence the production of metabolites by *Bacillus* spp. Also, several studies have proposed that LPs are co-produced and active in a synergistic way, for example, surfactin with iturin, surfactin with fengycin, and iturin with fengycin (Ongena and Jacques 2008; Jacques 2011).

8 Conclusions

The genus *Bacillus* includes many species with the potential to produce LP biosurfactants of great industrial, biotechnological, and environmental interest. This is why many studies are conducted to search for new biosurfactant-producing *Bacillus* strains, their identification, and the culture conditions that can reduce LP production costs. Bioconversion of industrial wastes into biosurfactants has the potential to be a source of new materials and can convert industrial waste into commercial products. Our investigation confirms that replacing traditional microbiological media with agro-industrial wastes as substrates for biosurfactant production by three *Bacillus* has great potential. Moreover, this will reduce many management problems related to the processing of industrial waste. It has been also shown that these three *Bacillus* spp. grown on agro-industrial wastes, and their cell-free supernatants as a source of biosurfactants, had antifungal activities. Natural bacteria like *Bacillus*, capable of suppressing pathogens and maintaining their population by competing against harmful microorganisms, could be successfully utilized as biopesticides in agricultural biotechnology. Finally, these *Bacillus* spp. grown on agro-industrial wastes may have the potential for application as biopesticides to control plant diseases.

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Production of Biosurfactants Using Eco-friendly Microorganisms

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Abstract

Pathogenicity of biosurfactant-producing microorganisms is currently raising some health, safety and environmental concerns. As a result, the industrial-scale production and application of biosurfactants as potential alternatives to the synthetic one is still an unachieved task. The production of biosurfactants using nonpathogenic/recombinant strains requires more attention and investigation for some advantages that includes the discovery of non-toxic biosurfactants suitable for all industrial applications, identifying new biosurfactant congeners with better inherent surface-active properties compared to that from pathogens and synthetic ones and the synthesis of biosurfactant without complex metabolic regulations. Although a number of nonpathogenic/recombinant, eco-friendly biosurfactant-producing strains have been documented, there is need for more research in this area focusing especially on improved biosurfactant production by these strains using optimisation processes and the discovery of new nonpathogenic/recombinant strains using molecular techniques for future sustainability.

Keywords

Bioremediation • Biosurfactants • Recombinant strains • Rhamnolipids

1 Introduction

Biosurfactants are surfactants of microbial origin which can be synthesised by several identified microorganisms including bacteria, yeast and fungi. Originally, the majority of the surfactants used in the industries today are petrochemical based, and these surfactants are not only partially biodegradable but are also toxic to living organisms and have contributed to a wide range of environmental hazards. In addition, the production of these petrochemical-based surfactants contributes to the depletion of the world's non-renewable petrochemical resources. It has been reported that the worldwide production of

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synthetic surfactants is over 15 million tons per year (Van-Bogaert et al. 2007) that are employed in different industrial sectors ranging from food processing, pharmaceutical, cosmetics, detergent production, environmental cleanup (bioremediation) and enhanced oil recovery. According to Reznik et al. (2010), the worldwide production of synthetic surfactants per annum was 13 million tons in 2008, increased by 2 % in 2009 resulting to annual turnover of US$ 2,433 billion. The market was then expected to experience a continuous growth by 28 % from 2009 to 2012 and thereafter increase approximately by 3.5–4 %. However, biosurfactants have been reported by many researchers to have a variety of advantages over the petrochemical-based or synthetic surfactants. They display excellent surface activity and emulsification properties with very low toxicity and higher biodegradability features. They have also been found to be very effective at low concentrations and over a wide range of environmental conditions such as pH, temperature, salinity, alkalinity and acidity. Other important characteristics of these biomolecules include better environmental compatibility, lower critical micelle concentration, higher selectivity, specific activity and ability to be synthesised from renewable low-cost resources (Desai and Banat 1997; Oliveira et al. 2009; Rahman and Gakpe 2008).

These biosurfactants due to their amphiphilic properties have found potential application in an extremely wide variety of industrial process involving emulsification, foaming, detergency, wetting, dispersing or solubilisation. In addition, biosurfactants are currently being used as antibacterial, antifungal, antiviral, antioxidant, moisturisers and antiradical and stabilising agents in the production of various industries, products ranging from cosmetics, food, pharmaceutical, agriculture and detergents. Their ability to stimulate dermal fibroblast metabolism and support healthy skin physiology has also put them on the front line as potential raw material for cosmetics such as deodorants, facial cosmetics, lotions, eye shadow, skin smoothing and anti-wrinkle and anti-ageing products (Rosenberg and Ron 1999; Banat et al. 2000; Rahman et al. 2002).

The commercial viability of biosurfactants as alternatives to the synthetic surfactants has been greatly hindered due to limiting technical and economic factors including high substrate cost (Mukherjee et al. 2006), low product yield (Maneerat 2005; Thavasi et al. 2011b), product mixtures resulting in high-cost downstream recovery/purification processes (Heyd et al. 2008) and the type of producing strains (Reiling et al. 1986). Most biosurfactant-producing organisms are pathogenic and difficult to handle in large-scale industrial processes (Gunther et al. 2005; Toribio et al. 2010). In this review, after a discussion of the pathogenicity of biosurfactant, we focus on the state of the art of production of biosurfactants from nonpathogenic eco-friendly organisms and on the heterologous production by recombinant strains.

2 Biosurfactant Production Using Pathogenic Organisms and Health and Safety Issues

The development of biosurfactant production in nonpathogenic organisms is a current challenge that is receiving increased attention in order to avoid pathogenicity and complex metabolic regulations (Dusane et al. 2010) especially in rhamnolipid synthesis by *Pseudomonas aeruginosa* and to screen for novel product spectra (Müller et al. 2012). Application of crude biosurfactants from pathogens in industrial and environmental applications is largely unacceptable especially in the cosmetics, health and food sectors due to the potential presence of toxins and pigments (Nicas and Iglewski 1985).

2.1 Rhamnolipids

Rhamnolipids are widely studied glycolipid biosurfactants synthesised in large quantities by different strains of *P. aeruginosa*. As biosurfactants, rhamnolipids play an important role for the producing organism, displaying useful physico-chemical, physiological (Zhong et al. 2007) and antimicrobial functions on other living systems (Bergstrom et al. 1946; Abalos et al. 2001). Excellent emulsifying properties relative to synthetic surfactants make rhamnolipids viable alternatives due to their efficacy at low concentrations and their inherent biodegradability (Ron and Rosenberg 2002; Calvo et al. 2009; Rahman et al. 2003), for example, the bioremediation of hydrocarbon-polluted sites (Thavasi et al. 2011a; Jorfi et al. 2013) and in enhanced oil recovery (Amani et al. 2013). However, the industrial-scale application of *P. aeruginosa* strains for rhamnolipid production is an unrealistic task: this organism is an opportunistic human pathogen (Rahman et al. 2010) and is responsible for infectious diseases in immune-compromised individuals (Goethals et al. 2001). Addressing these safety issues renders *P. aeruginosa*-derived biosurfactant uneconomical (Ochsner et al. 1995; Tuleva et al. 2002) and unsafe for industrial processes.

The cultivation of *P. aeruginosa*, a Gram-negative bacterium belonging to the taxonomical class of Gammaproteobacteria and the family Pseudomonadaceae, has been well studied, and the genome of strain PAO1 has been fully sequenced and annotated. The organism is found in various habitats including water, soil, plants and air, is resistant to a variety of antibiotics (Tummler et al. 1991) and is the main causative organism for cystic fibrosis and nosocomial infections. The pathogenicity of *P. aeruginosa* in cystic fibrosis was confirmed by Kownatzi et al. (1987) who discovered rhamnolipids of up to 8 µl/ml in the sputum of *P. aeruginosa*-colonised cystic fibrosis patients. Read et al. (1992) also showed the presence of 65 µl/ml rhamnolipid in the secretions of a lung removed from a cystic fibrosis patient. The cytotoxic and haemolytic effects of crude rhamnolipids from other rhamnolipid producers have been demonstrated by researchers (Häussler et al. 1998, 2003; Rahman et al. 2010). Rhamnolipids have been shown to be heat-stable haemolysins having haemolytic activity on various erythrocyte species (Fujita et al. 1988; Johnson and Boese-Marrazzo 1980) and cytotoxic at high concentration to immune cells (Bjarnsholt et al. 2005; Jensen et al. 2007).

Rhamnolipids from *P. aeruginosa* stimulate the release of allergy and inflammatory mediators from the mast cells such as histamine, serotonin and 12-hydroxyeicosatetraenoic acid (Bergmann et al. 1989; McClure and Schiller 1992; König et al. 1992; Cosson et al. 2002; Andrä et al. 2006). Furthermore, rhamnolipids have been associated with several dysfunctions in the lungs and the entire respiratory tract. These include inhibition of ciliary function, damage to bronchial epithelium, alteration of respiratory epithelial ion movement and induction of the release of mucus conjugates from human bronchial mucosa, and very recently it has been reported that rhamnolipid synthesis is an important prerequisite for the invasion of *P. aeruginosa* into human respiratory epithelial cells (Stutts et al. 1986; Graham et al. 1993; Fung et al. 1995; Zulianello et al. 2006). *P. aeruginosa* has been classified as a biosafety level 2 organism due to the health and safety issues associated with the organism as well as the cytotoxic properties of their rhamnolipid species.

These safety concerns inhibit the economic viability of large-scale rhamnolipid production from *P. aeruginosa*. Such viability is further compromised by the involvement of complex quorum sensing and transcriptional mechanisms (Soberón-Chávez et al. 2005). Furthermore, the purification and treatment of the rhamnolipid yield from *P. aeruginosa* are also costly at the industrial scale, and it is important to note that rhamnolipids produced from *P. aeruginosa* fermentation cannot be considered as safe raw materials in the production of food, pharmaceutical and cosmetics products. Thus there is need to explore for new nonpathogenic natural rhamnolipid producers. A survey of biosurfactants produced by pathogenic strains is presented in Table 1 together with their pathogenesis in humans, plants and animals.

Rhamnolipids are also produced by *Burkholderia* species; some of these organisms such as *B. cepacia* have been identified as human pathogens. This organism capable of producing a biosurfactant exhibiting a surface tension value of 45.7 mN m^{-1} (Yalçin and Ergene 2010) has been

Table 1 Biosurfactant production by pathogenic bacterial strains

Biosurfactants	Pathogenic producers	Pathogenicity	References
Rhamnolipids	*Pseudomonas aeruginosa*	Cystic fibrosis, respiratory infections, multidrug resistant	Thavasi et al. (2011b), Jorfi et al. (2013), Kownatzi et al. (1987)
	Burkholderia cepacia	Lung infection, pneumonia	St Denis et al. (2007), Yalçin and Ergene (2010)
	Burkholderia pseudomallei	Melioidosis	Andrea et al. (2008), Howe et al. (2006)
	Burkholderia plantarii	Fire blight disease of apple and pear trees	Hörmann et al. (2010), Mitchell and Teh (2005)
	Burkholderia glumae	Rot of rice grains and seedlings	Urakami et al. (1994), Costa et al. (2011)
	Renibacterium salmoninarum	Bacterial kidney disease of salmonid fish	Christova et al. (2004), Wiens et al. (2008)
Glycolipids	*Pantoae agglomerans*	Bacteraemia, wound infection, arthritis, infection of soft tissues bones and joints in children	Vasileva-Tonkova and Geshava (2007), Jacobucci et al. (2009), Koo et al. (2006)
	Nocardia otitidiscaviarum	Pulmonary nocardiosis, brain abscess, actinomycetoma	Vyas and Dave (2011), Pelaez et al. (2009), Chi et al. (2013)
	Alcaligenes faecalis	Post-operative endophthalmitis, peritonitis	Kaliaperumal et al. (2006), Kahveci et al. (2011), Bharali et al. (2011)
Trehalose mycolates	*Acinetobacter calcoaceticus* IMV B-7241	Urinary tract infections, respiratory tract infections, post-operative infections	Pirog et al. (2013), Pal and Kale (1981)
Phospholipids	*Klebsiella pneumoniae*	Haemorrhage/necrosis, upper and lower respiratory tract infections, osteomyelitis, UTI, diarrhoea, wound infection, meningitis, bacteraemia, septicaemia, ankylosing spondylitis	Jamal et al. (2012), Rashid and Ebringer (2007), Ryan and Ray (2004), Limbago et al. (2012)
Lipopeptides	*Serratia marcescens*	Urinary tract infections, nosocomial bacteraemia, meningitis, endocarditis	Ibrahim et al. (2013), Anyanwu et al. (2010), Körner et al. (1994)
	Inquilinus limosus KB3	Cystic fibrosis, multidrug-resistant lung infections	Saimmai et al. (2013), Wellinghausen et al. (2005), Hayes et al. (2009)
Heteropolysaccharides	*Cronobacter sakazakii*	Bacteraemia, meningitis, necrosis, enterocolitis, contamination of infant formula	Lai (2001), Jain et al. (2012), CDC (2002)

associated with lung infections and pneumonia in humans of all ages (St Denis et al. 2007). Similarly, *B. pseudomallei*, a biosurfactant producer (Howe et al. 2006), is a facultative intracellular pathogen, although the mechanism of its intracellular survival is yet unknown. This pathogen is the causative agent of melioidosis, an infectious disease endemic to Southeast Asia, northern Australia and tropical and subtropical regions (Andrea et al. 2008). The di-rhamnolipids from *B. pseudomallei* have been found to exhibit serious cytotoxic effects on macrophages non-phagocytic and phagocytic cell lines (Sierra 1960; Kharazmi et al. 1989; Johnson and Boese-Marrazzo 1980; Fujita et al. 1988). Di-rhamnolipids have also been reported to be cytolytic to human monocyte-derived macrophages and can inhibit the phagocytic response of macrophages even at low concentration.

Burkholderia plantarii DSM 9509 has been demonstrated to be the most prominent rhamnolipid producer. According to Hörmann et al. (2010) and Walter (2009), *B. plantarii* DSM 9509 produced Rha2-C_{14}-C_{14} congeners that reduced the surface tension of water to 29.4 mN m^{-1} with critical micelle concentrations (CMC) between 15 and 20 mg L^{-1}. The maximum rhamnolipid concentration during 0.5 L bioreactor cultivation was 45.75 mg L^{-1}. However, this organism is a plant pathogen responsible for the fire blight disease of apple and pear trees, and the di-rhamnolipid from *B. plantarii* is an endotoxin (Mitchell and Teh 2005). Costa et al. (2011) also showed rhamnolipid concentration of 1 g L^{-1} in *B. glumae* AU6208 at 34 °C, 2 % canola oil and 100 mM urea, but this organism is toxic to rice grain and seedlings.

Renibacterium salmoninarum is a diplobacillus fastidious bacterium identified as potent biosurfactant producer on n-hexadecane as a carbon source (Christova et al. 2004). It is also interesting to know that this bacterium produces the two typical rhamnolipid as in *P. aeruginosa* and has the potential of being useful in natural degradation of hydrophobic pollutants due to its high cell hydrophobicity. Despite these promising characteristics, this organism has raised a significant ecological concern. It causes a haemorrhagic infection known as bacterial kidney infection (BKD) or salmonid kidney disease in young salmonid fish (Wiens et al. 2008).

2.2 Glycolipids and Trehalose Mycolates

Glycolipid production by other known pathogens has been reported. The Antarctic facultative anaerobe *Pantoea agglomerans* is a biosurfactant producer with hydrocarbon compounds (hexane, kerosene and paraffin) as growth substrates (Vasileva-Tonkova and Gesheva 2007; Jacobucci et al. 2009). *Pantoea* sp. has also been reported as producers of high levels of exopolysaccharides with maximum production of 21 g L^{-1} in 24 h on glucose, fructose and sucrose. However, *Pantoea* sp. have recently been consistently linked with infections in humans (wound, blood, soft tissue, bone and urinary tract infections) and plants (Da Baere et al. 2004; Fullerton et al. 2007; Kratz et al. 2003). These organisms have also been associated with bacteraemia outbreaks in many patients through their contact with contaminated cotton pledgets (Koo et al. 2006; Silvi et al. 2013).

Production of glycolipid biosurfactant has been reported for *Nocardia otitidiscaviarum*, a marine strain. The biosurfactant possessed increased cell hydrophobicity, making it very potent for bioremediation of oil-polluted sites (Vyas and Dave 2011). Recently, these bacterial species have been identified as causative agents in a couple of clinical infections ranging from pulmonary nocardiosis, brain abscess and actinomycetoma (Pelaez et al. 2009; Chi et al. 2013).

Alcaligenes faecalis has been reported by Bharali et al. (2011) as a producer of biosurfactant exhibiting excellent surface activity using different hydrocarbon substrates. However, *A. faecalis* is a known pathogen infecting human and domesticated birds such as chickens and turkeys (Simmons et al. 1981). Kaliaperumal et al. (2006) reported *A. faecalis* as the causative agent in post-operative endophthalmitis in human eye resulting in symptoms such as swelling of the eyelid, redness and permanent loss of vision. In addition, Kahveci et al. (2011) have reported a link between the developments of peritonitis to contamination of catheters by *A. faecalis*. The pathogenic mechanism of this microbe is largely unknown.

Rosenberg et al. (1989) identified *Acinetobacter calcoaceticus* A2 as a producer of an extracellular anionic surfactant referred to as biodispersan or emulsan which is a high molecular weight biosurfactant. Pirog et al. (2009) have also reported the production of low molecular weight biosurfactant by *A. calcoaceticus* IMV B-7241 which was confirmed to be trehalose mycolates by enzymatic studies. *A. calcoaceticus* has been described as a commensal in humans but is also responsible for many clinical infections as an opportunistic pathogen. These infections include urinary tract infection, pneumonia, respiratory tract infection and post-operative infections (Pal and Kale 1981).

2.3 Phospholipids and Lipopeptides

Biosurfactant production by a prominent member of the *Klebsiella* genus known as *Klebsiella pneumoniae* has been identified. According to Jamal et al. (2012), *Klebsiella pneumoniae* WMF02 showed increased biosurfactant production after medium optimisation with a maximum yield of 85 g L^{-1} and surface tension reduction of 25.70 mN m^{-1} compared to the non-optimised medium (36.2 mN m^{-1}). This organism has shown the promising potential of a good biosurfactant producer, but its application will involve certain health and safety risks. *K. pneumoniae* is a significant human pathogen causing different destructive effects to the human upper and lower respiratory tracts if inhaled which includes pneumonia and bronchitis. Other notable infections caused by this organism include diarrhoea, wound infections, osteomyelitis, meningitis, bacteraemia and septicaemia (Ryan and Ray 2004). Recently, these bacterial strains have been reported as resistant to antibiotics especially carbapenem antibiotics (Limbago et al. 2012). They have also been associated with a chronic inflammatory spinal and large joint arthritic condition known as ankylosing spondylitis (AS) affecting young-aged males (Rashid and Ebringer 2007).

Serratia marcescens is a multidrug-resistant pathogen causing infections in different hosts ranging from plants, animals and humans. This bacterium produces a lipopeptide biosurfactant known as serrawettin (Dusane et al. 2011). The biosurfactant is very potent and has been reported in literature to reduce the surface tension of water from 72 to 37 mN m^{-1}, emulsifying kerosene and diesel with a maximum emulsion index of 72 % and 40 %, respectively (Wei et al. 2004). Ferraz et al. (2002) also reported biosurfactant production by *S. marcescens* strains. The biosurfactant reduced the surface tension of the culture medium from 64.54 to 29.57 mN m^{-1}, while the crude biosurfactant reduced the surface tension of water from 72 to 28.70 mN m^{-1}. However, this bacterium cannot stand the chance of being used for industrial-scale production of biosurfactant due to its pathogenicity. Most importantly, serrawettin biosurfactant is a virulence factor, and *S. marcescens* strains have been linked with a number of infections including nosocomial infections bacteraemia, urinary tract infections, meningitis and endocarditis (Anyanwu et al. 2010; Körner et al. 1994). According to a report by the Centers for Disease Control in the USA, *S. marcescens* was confirmed as the main causative organism in the Alabama hospital outbreaks that happened in 2011 affecting 19 patients with 10 deaths.

Saimmai et al. (2013) identified a Gram-negative bacterium *Inquilinus limosus* KB3 capable of producing lipopeptide biosurfactant using palm oil cake as a carbon source. The biosurfactant reduced the surface tension of water from 72 to 25.5 mN m^{-1} with a maximum yield of 5.13 g L^{-1} and CMC at 9 mg L^{-1}. The genus *Inquilinus* was first defined in 2002 based on the molecular analysis of 51 unknown cystic fibrosis isolates. This group of organisms is unrelated to *P. aeruginosa* and *B. cepacia. Inquilinus limosus* has been reported as a multidrug-resistant pathogen and has been isolated from the lungs of cystic fibrosis patients (Wellinghausen et al. 2005; Hayes et al. 2009).

2.4 Heteropolysaccharides

Jain et al. (2012) reported the production of a heteropolysaccharide biosurfactant by an alkaliphilic bacterium, *Cronobacter sakazakii*, formerly known as *Enterobacter sakazakii*. The biosurfactant comprised of total sugars (73.3 %), reducing sugars (1.464 %), protein (11.9 %), uronic acid (15.98 %) and sulphate (6.015 %). The monosaccharide moieties in this biosurfactant were revealed by GC-MS as glucose (14 %), mannose (24 %), galactose (14 %), xylose (20 %) and arabinose (19 %). The extracted biosurfactant from this bacterium efficiently emulsified aliphatic and aromatic hydrocarbons forming stable emulsions in the presence of xylene, cyclohexane, cyclooctane, toluene, carbon tetrachloride, dichloromethane, cottonseed, jojoba and groundnut oil at 1 mg mL^{-1}. The inherent properties of the biosurfactant make it a potential candidate for bioremediation of oil and hydrocarbons. However, *Cronobacter sakazakii* is a Gram-negative pathogenic bacterium (Lai 2001), reported as the cause of meningitis bacteraemia, necrosis and enterocolitis in infants. Infections have been associated with the use of powdered infant formula contaminated with the bacterium (Bowen and Braden 2006; CDC 2002).

The industrial application of pathogenic biosurfactant producers is clearly problematic based on the pathogenic effects of the organisms and their products on humans and animals. Additionally, some of these pathogens exhibit multidrug resistance and have recorded high mortality rates from their associated diseases. Therefore, they are now considered as potential biological warfare agents and as such are not potential biological models for industrial biotechnological processes (Walter 2009). For this combination of security and health reasons, there is a need to develop industrial processes based on nonpathogenic biosurfactant-producing strains.

3 Biosurfactant Production Using Nonpathogenic Organisms

Recently, due to the health and safety issues associated with some biosurfactant-producing strains, research towards identifying new nonpathogenic producers and biotechnological production of biosurfactants using recombinant/mutant strains has been given more attention. The advantages of using nonpathogenic organisms may include production of biosurfactants with various congener species, including the possibility of biosurfactants with less or no cytotoxic effects on living cells, and disconnection of their synthetic pathways from complex mechanisms such as quorum sensing in rhamnolipid synthesis. Nonpathogenic natural producer strains and their biosurfactant types identified to date have been reported (Table 2).

3.1 Rhamnolipids

Novel natural producers of rhamnolipids identified as *P. clemancea* nov. and *P. teessidea* nov. have been reported by Rahman et al. (2010). Rhamnolipid production can be achieved with *Pseudomonas* sp. other than *P. aeruginosa. Pseudomonas fluorescens* is a nonpathogenic Gram-negative, rod-shaped bacterium that has been found to produce biosurfactants as well as useful enzymes (cellulase, pectinase), self-defence factors (hydrogen cyanide), siderophores (pyochelin, pyoverdine), antibiotics (pyrrolnitrin, pyoluteorin) and 2,4-diacetylphloroglucinol, a molecule that can break down plant-derived carbohydrates, enhance host immune mechanisms and inhibit phytopathogens and bacteriophages (Stover et al. 2000; Paulsen et al. 2005). This organism has been reported as a degrader of certain environmental pollutants such as styrene, TNT and polycyclic aromatic hydrocarbons and as a result has been employed in many bioremediation processes.

The production of a thermostable rhamnolipid biosurfactant with excellent foaming and emulsifying stability by *P. fluorescens* was reported by Abouseoud et al. (2007). The biosurfactant was stable at 100 °C and retained its positive effect on surface tension (34–30 mN m^{-1}) even at high pH values. Stoimenova et al. (2009) isolated from industrial wastewater *P. fluorescens* HW-6 capable of producing rhamnolipid biosurfactants at relatively high levels on various carbon substrates including hexadecane, vegetable oil, mineral oil and glycerol. The culture supernatant of the strain exhibited a reduction in surface tension to 28.4 mN m^{-1}. Vasileva-Tonkova et al. (2006) also reported production of rhamnolipid biosurfactant by *P. fluorescens* HW-6 at concentrations of 14–20 g L^{-1} on hexadecane and interfacial tension of 35 mN m^{-1}, possessing a low critical micelle concentration value of 20 mg L^{-1}. Recently, a rhamnolipid exhibiting high antimicrobial

Table 2 Biosurfactant production using nonpathogenic bacteria

Biosurfactants	Microbial strains	Physico-chemical characteristics	References
Rhamnolipids	*Pseudomonas clemancea* nov.	Mesophilic	Rahman et al. (2010)
	Pseudomonas teessidea nov.	Mesophilic	Rahman et al. (2010)
	Pseudomonas fluorescens	Mesophilic	Abouseoud et al. (2007), Vasileva-Tonkova et al. (2006)
	Pseudomonas chlororaphis	Mesophilic	Gunther et al. (2005)
	Pseudomonas putida BD2	Mesophilic	Janek et al. (2013)
	Burkholderia thailandensis	Mesophilic	Dubeau et al. (2009)
	Enterobacter asburiae	Mesophilic	Hošaková et al. (2013)
	Thermus aquaticus	Thermophilic	Řezanka et al. (2011)
	Meiothermus ruber	Thermophilic	Řezanka et al. (2011)
	Enterobacter hormaechei PTCC 1799	Mesophilic	Rabiei et al. (2013)
	Tetragenococcus koreensis	Halophilic	Lee et al. (2005)
	Pseudoxanthomonas sp. PNK-04	Mesophilic	Nayak et al. (2009)
Glycolipids	*Streptococcus thermophilus* A	Thermophilic	Rodrigues et al. (2006a)
	Lactococcus lactis 53 *L. acidophilus*	Mesophilic	Rodrigues et al. (2006b), Tahmourespour et al. (2011)
	Lactobacillus coryniformis sp. *torquens* CECT 25600	Mesophilic	Gudiña et al. (2011)
	Lactobacillus paracasei sp. *paracasei* A20	Mesophilic	Gudiña et al. (2011)
	L. plantarum A14	Mesophilic	Gudiña et al. (2011)
	Leuconostoc mesenteroides	Mesophilic	Gudiña et al. (2011)
	Lactobacillus delbrueckii	Mesophilic	Thavasi et al. (2011a)
Trehalose lipids	*Rhodococcus erythropolis* IMV Ac-5017, *Rhodococcus* sp.	Mesophilic	Pirog et al. (2013), Mutalik et al. (2008)
	R. erythropolis ATCC 4277	Mesophilic	Pacheco et al. (2010)
	R. ruber Z25	Mesophilic	Zheng et al. (2009)
Lipopeptides/phospholipids	*Bacillus subtilis* DM-03, *Bacillus subtilis* DM-04	Mesophilic	Das and Murkherjee (2007)
	B. subtilis PT2	Mesophilic	Pornsunthorntawee et al. (2008)
	B. subtilis LB5a	Mesophilic	Nitschke and Pastore (2006)
	B. subtilis	Mesophilic	Cooper et al. (1981)
	B. lentus, *B. firmus*	Mesophilic	Ibrahim et al. (2013)
	Bacillus licheniformis		Biria et al. (2010)
	Selenomonas ruminantium	Mesophilic	Saimmai et al. (2013)
	Brevibacterium aureum MSA13	Mesophilic	Kiran et al. (2010)
	Corynebacterium kutscheri	Mesophilic	Thavasi et al. (2007)
	Corynebacterium alkanolyticum ATCC 21511	Mesophilic	Crosman et al. (2002)
Heteropolysaccharides	*Halomona* sp. BS4	Halophilic	Donio et al. (2013)
	Halomonas sp. TG39	Halophilic	Gutierrez et al. (2012)
	Halomona eurihalina	Halophilic	Calvo et al. (2002)

activities on both Gram-positive and Gram-negative pathogenic strains – *Listeria monocytogenes*, *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *E. coli*, *Salmonella typhimurium* and *Candida albicans* – has been reported to be produced from *Pseudomonas fluorescens* MFS03 (Govindammal and Parthasarathi 2013). The biosurfactant was effective as a surface and emulsifying agent and could have potential applications in the bioremediation of hydrocarbon-polluted sites. Rhamnolipid production was also reported for nonpathogenic *P. chlororaphis* (Gunther et al. 2005) and *P. putida* BD2 (Janek et al. 2013).

Dubeau et al. (2009) reported production of 0.4–1.5 g L^{-1} rhamnolipid by *Burkholderia thailandensis* at 34 °C with 4 % glycerol or canola oil in nutrient broth. *B. thailandensis* is a Gram-negative, mesophilic bacterium closely related to *B. pseudomallei* but rarely causes infections in humans or animals (Wuthiekanun et al. 1996; Smith et al. 1997; Lertpatanasuwan et al. 1999). The lethal inoculum size for *B. thailandensis* is approximately 1,000 times higher than that for *B. pseudomallei* (Joost Wiersinga et al. 2008). This organism does not require biosafety level 3 conditions and has no restriction on the use of antibiotics or resistance markers for its genetic manipulation. The organism is not considered as a biosecurity threat and therefore is a potential industrial tool (Haraga et al. 2008; Glass et al. 2006). Recently, Hošaková et al. (2013) identified *Enterobacter asburiae* as nonpathogenic rhamnolipid producers. Rezanka et al. (2011) reported on three novel rhamnolipid-producing organisms and were identified as *Thermus* sp., *Thermus aquaticus* and *Meiothermus ruber*. These organisms have been categorised as biosafety level 1 organisms and are not pathogenic to humans. Pantazaki et al. (2010) have reported the simultaneous production of polyhydroxyalkanoates (PHAs) and rhamnolipids by a nonpathogenic thermophilic bacterium *Thermus thermophilus* HB8 (DSM 579) cultivated in mineral salt medium at 75 °C using glucose and sodium gluconate as sole carbon sources. Other new nonpathogenic rhamnolipid producers include *Enterobacter hormaechei* PTCC 1799 (Rabiei et al. 2013), *Tetragenococcus koreensis* (Lee et al. 2005) and *Pseudoxanthomonas* sp. (Nayak et al. 2009).

3.2 Glycolipids

The *Streptococcus thermophilus* bacterium is used widely in the dairy industries for the production of yogurt and cheese and is considered as beneficial to health since it aids digestion of dairy products in lactose-intolerant individuals (Kiliç et al. 1996; Hutkins 2002; Taylor and Mitchell 2007). According to Busscher et al. (1997), a biosurfactant released from *S. thermophilus* was used in the control of fouling of heat-exchanger plates in pasteurisers because it inhibited the colonisation of other thermophilic and pathogenic strains of *Streptococcus* responsible for fouling. Two probiotic bacteria *Lactococcus lactis* 53 and *S. thermophilus* A have been identified as producers of cell-bound biosurfactants at the stationary growth phase in the presence of lactose and cheese whey as carbon substrates (Rodrigues et al. 2006a, b). A protein-containing biosurfactant from *Lactobacillus acidophilus* has also been demonstrated to reduce the adhesion and biofilm formation of *Streptococcus mutans* on glass slides (Tahmourespour et al. 2011), indicating that the treatment of teeth with this biosurfactant may be an alternative dental control for biofilm development and adhesion of the pathogens on teeth.

Similarly, Gudiña et al. (2011) investigated the production of cell-bound and excreted biosurfactant by three lactobacilli strains and *Leuconostoc mesenteroides* strains. The lactobacilli strains were *L. coryniformis* sp. *torquens* CECT 25600, *L. paracasei* sp. *paracasei* A20 and *L. plantarum* A14. The studies revealed a decrease in surface tension of the culture broth for all the strains after 72 h incubation and the surface tension ranged from 1.4 to 6.4 mN m^{-1}. The highest excreted biosurfactant rate was recorded for *L. paracasei* sp. *paracasei* A20 with surface tension of 6.4 mN m^{-1}. However, the level of cell-bound biosurfactant was found to be higher than that of excreted molecules for all the strains. This is in contrast to other microorganisms such as *Pseudomonas* and *Bacillus* that primarily excrete biosurfactant into their medium. Biosurfactant production has also been reported for nonpathogenic *L. delbrueckii* by Thavasi et al. (2011a). This organism showed maximum glycolipid production of 5.35 mg L^{-1} at 144 h incubation with peanut oil cake as substrate, but higher production was recorded after the stationary phase of growth. This was thought to be due to the release of cell-bound biosurfactant in the early stationary phase. In addition, *L. delbrueckii* could maximally degrade 61.25 % crude oil in the presence of fertilisers; hence the organism is both a biosurfactant producer and a hydrocarbon degrader. Lactobacilli are probiotics and nonpathogenic organisms designated "Generally Recognized as Safe" (GRAS) by the American Food and Drug Administration (FDA). The organism's ability to utilise lactose instead of glucose via an alternative metabolic pathway for biosurfactant synthesis has proven them as ideal hosts for biotechnological techniques especially metabolic engineering towards large-scale production of cell-bound biosurfactants (Rodrigues et al. 2006c). Although the biosurfactant yield by these organisms is low, optimisation of culture conditions may improve their production.

Rhodococcus sp. are a group of aerobic, nonsporulating, Gram-positive bacteria that can be found in a wide range of environments. They are usually considered as experimentally advantageous due to their high growth rate and simple developmental cycle. These organisms are effective for the degradation of aromatic hydrocarbons, production of bioactive steroids, bio-desulphurisation of fossil fuel and bioconversion of waste products to valuable compounds (McLeod and Eltis 2008). Strains of these species have been reported as biosurfactant producers including *R. erythropolis* IMV Ac-5017 (Pirog et al. 2013) and *Rhodococcus* sp. MTCC 2574 (Mutalik et al. 2008). Furthermore, Pacheco et al. (2010) have identified *Rhodococcus erythropolis* ATCC 4277 is a producer of a biosurfactant exhibiting excellent enhanced oil desorption from an oil shale. *Rhodococcus ruber* Z25 identified by 16 rDNA sequencing produced cell growth-associated biosurfactants on n-hexadecane with maximum yield of 13.34 g L^{-1} at 44 h (Zheng et al. 2009). *Rhodococcus* spp. produce surface-active trehalose lipids, and reports have shown that these surface-active compounds present interesting physico-chemical and biological properties. Trehalose lipids can significantly reduce the surface tension of water from 70 mN m^{-1} to 30.8 mN m^{-1} (Mutalik et al. 2008) and can form microemulsions (Zaragoza et al. 2013). In addition, trehalose lipids have been reported to inhibit protein kinase activity in vivo (Isoda et al. 1997) and induce the cell lysis of *E. coli* IEM-1 as well as the vegetative and spore cells of *Bacillus subtilis* BT-2 (Pirog et al. 2013). They have also been found useful in soil bioremediation and microbial enhanced oil recovery (Philip et al. 2002; Bell et al. 1998). This group of bacteria is considered as an ideal host for biotechnological production of non-toxic biosurfactant on industrial scales because of their genetic and catabolic diversity (associated presumably with their large chromosome and three large linear plasmids (van der Geize and Dijkhuizen 2004; McLeod et al. 2006). These organisms are usually nonpathogenic with only two species *R. fascians* and *R. equi* (Goethals et al. 2001) identified as plant and animal pathogens as well as causing infections in immunecompromised individuals. Nonpathogenic biosurfactant producers and their biosurfactants are shown in Table 2.

3.3 Lipopeptides and Phospholipids

Biosurfactants other than glycolipids produced by nonpathogenic organisms have been documented in literature. *Bacillus subtilis* is a ubiquitous Gram-positive rod-shaped bacterium, found commonly in water, soil and air, and contributes to nutrient cycling in the environment. This organism is industrially useful as it is one of the most widely used bacteria in the production of enzymes (amylases, proteases, inosine, ribosides and amino acids) and speciality chemicals including biosurfactants (Erikson 1976). This bacterium has also been shown to produce a variety of antibacterial (Katz and Demain 1977) and antifungal (Korzybski et al. 1978) compounds including difficidin and oxydifficidin with wide spectrum of antibiotic activities against aerobic and anaerobic bacteria (Zimmerman et al. 1987). It has been used as a fungicide because it has the inherent ability to colonise root systems and to inhibit the growth of fungal plant pathogens (Kimura and Hirano 1988; Loeffler et al. 1986). Although *B. subtilis* has been associated with outbreaks of food poisoning (Gilbert et al. 1981; Kramer et al. 1982) and human infections especially in hospitalised patients with surgical wounds, breast cancer and leukaemia (Logan 1988), the exact nature of its involvement in infections has not been established. However, a literature review by Edberg (1991) revealed that *B. subtilis* does not produce significant quantities of extracellular enzymes or virulence factors that would predispose it to cause infection. In addition, Ihde and Armstrong (1973) reported that *B. subtilis* is an organism with low virulence. The organism has therefore been classified as neither a human (Edberg 1991) nor plant (Claus and Berkeley 1986) pathogen. According to the National Institutes of Health (NIH) guidelines for research involving recombinant DNA molecules (US Department of Health and Human Services 1986) and the European Federation of Biotechnology guidelines, *B. subtilis* is considered a class 1 containment agent, and their industrial use in fermentation processes presents low risk of adverse effects to human health and environment. *Bacillus subtilis* produces an effective and active cyclic lipopeptide biosurfactant known as surfactin (Cooper et al. 1987; Peypoux et al. 1999). Das and Mukherjee (2007) reported the production of lipopeptide surfactants by two strains *Bacillus subtilis* DM-03 and *Bacillus subtilis* DM-04 on potato peels using both submerged and solid-state fermentation techniques. Production of biosurfactant with enhanced surface tension reduction of 26 mN m^{-1} and lower CMC of about 25 mg L^{-1} has been reported for *B. subtilis* PT2 strain (Pornsunthorntawee et al. 2008). Similarly, *B. subtilis* LB5a decreased surface tension to a minimum value of 26.6 mN m^{-1} with CMC of 33 mg L^{-1} (Nitschke and Pastore 2006). Cooper et al. (1981) had earlier reported production of lipopeptide biosurfactants by *B. subtilis* strains with a minimum surface tension of 25 mN m^{-1} and a CMC of 25 mg L^{-1}. Surfactin has been demonstrated as one of most effective biosurfactants because of its high surface activity, efficiency in bioremediation and in situ microbial enhanced oil recovery (Mulligan 2005; Awashti et al. 1999; Besson and Michel 1992). Therefore, the production of biosurfactants from *B. subtilis* has the potential for large-scale bio-industrial development. Nonpathogenic lipopeptide-producing bacteria identified as *Bacillus lentus* and *B. firmus* have also been reported by Ibrahim et al. (2013) and Joshi et al. (2013).

Bacillus licheniformis is an important producer of lipopeptide biosurfactants (Biria et al. 2010) and has been used in industrial fermentation processes for over a decade for the production of several enzymes, antibiotics and special chemicals (Gherna et al. 1989; Eveleigh 1981). Although these *Bacillus* species have been reported to be associated with human infections, these occurred only in immunosuppressed individuals following trauma and other predisposing factors. Therefore, according to the Biotechnology Program under the Toxic Substances Control Act under the US EPA (1997), *B. licheniformis* is classified as a nonpathogen and is not toxigenic. Furthermore, the National Institutes of Health and European Federation of Biotechnology guidelines have also placed this organism as a class 1 containment agent (Frommer et al. 1989). Nonpathogens other than *Bacillus* sp. such as *Selenomonas ruminantium* (Saimmai et al. 2013) and *Brevibacterium aureum* MSA13 (Kiran et al. 2010) have also been identified for the production of lipopeptide biosurfactants.

Corynebacterium sp. is Gram-negative, catalase-positive, rod-shaped facultative anaerobic bacterium. Most members of this genus are nonpathogenic and industrially useful as they are known for the synthesis of amino acids, nucleotides and enzymes, for the bioconversion of steroids and for the degradation of hydrocarbons and are used in cheese ageing (Seidel et al. 2007; Natsch et al. 2005). The production of glycolipopeptide and phospholipid biosurfactants has been reported for two nonpathogenic species such as *C. kutscheri* and *C. alkanolyticum* ATCC 21511, respectively (Thavasi et al. 2007; Crosman et al. 2002).

3.4 Heteropolysaccharides

Halomonas are a group of Gram-negative, nonsporing bacteria usually found in many different extreme water and soil environments, mainly saline, hypersaline or alkaline ecosystems. They belong to the family of Halomonadaceae and are classified as Gammaproteobacteria currently made up of 10 genera. They are considered to be nonpathogenic (von Graevenitz et al. 2000) and are potential industrial microbes due to their ability to synthesise microbial exopolysaccharides. Levan and mauran are examples of microbial polysaccharides from *Halomonas* spp. with a considerable market due to their exceptional performance at extreme industrial conditions (Poli et al. 2009; Llamas et al. 2006.) Although a few strains have been associated with certain human infections and contamination in a dialysis centre (Stevens et al. 2009), several *Halomonas* species have been classified as nonpathogens and also identified as biosurfactant producers. According to Donio et al. (2013), *Halomonas* sp. BS4 isolated from solar salt works has produced a heteropolysaccharide biosurfactant that suppressed the proliferation of mammary epithelial carcinoma cells by 46.77 % at 25 µL concentration. In addition, the pure biosurfactant exhibited antibacterial activity on *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* and *Salmonella typhi* and antifungal activity on *Aspergillus niger*, *Fusarium* sp., *Aspergillus flavus* and *Trichophyton rubrum*. The biosurfactant was also found to display antiviral activity on white spot syndrome virus at high percentage (60, 80 and 100 %) and effectively suppressed the pathological effect of the virus. Gutierrez et al. (2013) have also shown the significance of exopolysaccharide (EPS) produced from marine *Halomonas* sp. TG39 in trace metal biogeochemical cycling. Calvo et al. (2002) have also documented the production of EPS from *Halomona eurihalina*. This research revealed that EPS from *Halomonas* contained high levels of K, Ca, Mg and several trace metals such as Zn, Cu, Fe and metalloid Si and has the specific ability to bind Ca, Si, Fe, Mn, Mg and Al in marine sediments. Furthermore, the growth of marine diatom *Thalassiosira weissflogii* was enhanced in the presence of purified EPS or to marine sediments exposed to EPS, indicating that the trace metals bound to the EPS become biologically available for the diatoms to utilise for growth. This bacterium therefore has the potential for the biotechnological development of safe antimicrobial and anticancer drugs as well as eco-friendly environmental products.

4 Production of Biosurfactant by Recombinant Strains

Pathogenicity may be avoided by the expression of biosurfactant production in heterologous microbial strains and mutants. Rhamnolipids can be synthesised by nonpathogenic heterologous strains provided with the rhamnosyltransferase genes *rhlA*, *rhlB and rhlC*. According to Cha et al. (2008), nonpathogenic heterologous *P. putida* 1067 (pNE2) expressing the *rhlABRI* gene from pathogenic *P. aeruginosa* EMS1 cultured in the mineral salt medium for 7 days in the presence of 2 % soybean oil as the sole carbon source exhibited rhamnolipid productivity that was greater than that of *P aeruginosa* EMS1. Scope remains for optimisation of the production conditions potentially leading to increased biosurfactant production making this organism useful for industrial biosurfactant production. Furthermore, Ochsner et al. (1995) investigated the heterologous expression of *rhlAB in P. fluorescens*, *P. putida* and *E. coli*. The results showed 0.25 g L^{-1}, 0.6 g L^{-1} and no rhamnolipid, respectively, for the organisms, but Wang et al. (2007) have reported rhamnolipid production in *E. coli* BL21 with the expression of *rhlAB* operon (Table 3).

In another study, *rmlBDAC* operon involved in dTDP-L-rhamnose biosynthesis was introduced into *E. coli* W3110 in addition to *rhlAB* operon, and a total final concentration of 120.6 mg L^{-1} mono-rhamnolipid was achieved using glucose as

Table 3 Recombinant biosurfactant producers

Biosurfactants	Microbial strains	References
Rhamnolipids	*Pseudomonas putida* 1067(PNE2)	Cha et al. (2008)
	Pseudomonas fluorescens	Ochsner et al. (1995)
	E. coli W3110, *E. coli* HB101	Cabrera-Valladares et al. (2006)
	P. putida KT2440	Wittgens et al. (2011)
	Burkholderia kururiensis KP23(T)	Tavares et al. (2013)

carbon substrate (Cabrera-Valladares et al. 2006). Similarly, recombinant *E. coli* HB101 was also detected to produce 52 mg L^{-1} rhamnolipid with oleic acid as substrate (Cabrera-Valladares et al. 2006). A successful development of a non-pathogenic host capable of producing mono-rhamnolipids using glucose as substrate has been reported by Wittgens et al. (2011). In this study, the synthesis of mono-rhamnolipid independent from biomass formation was done using *P. putida* KT2440 expressing the *rhlAB* genes from *P. aeruginosa* PA01. A sevenfold increase in the final rhamnolipid concentration from 0.22 to 1.5 g L^{-1} was recorded after genetic optimisation of the strain. The engineered strain exhibited some advantages compared to rhamnolipid production in *P. aeruginosa*. Firstly, these strains are nonpathogenic and can appropriately substitute the opportunistic pathogenic *P. aeruginosa*. Secondly, rhamnolipid production in the recombinant strains was void of the complex quorum sensing regulation. However, the production rate in the recombinant strain was about two thirds of that usually obtained in optimised fermentation with *P. aeruginosa* although there remains the possibility of increasing the production rate by increasing the availability of activated rhamnose in the medium. More importantly, compared to *P. aeruginosa*, it is the utilisation by the recombinant strains of glucose instead of hydrophobic substances. Glucose as a carbon substrate is relatively cheap and is applied in many biotechnological processes (Blank et al. 2008). Furthermore, these recombinant strains capable of substituting *P. aeruginosa* can utilise hydrophilic substances, reaching a maximum rhamnolipid concentration in medium after 1–2 days. This is compared with the wild-type strain of *P. aeruginosa* PA01 that although it exhibits higher rhamnolipid production, it requires 1–3 days to reach their maximal levels. This reduction in process time exhibited by recombinant strains enhances the space-time yield for the process (Wittgens et al. 2011). According to this investigation, the engineered *P. putida* KT2440 is the second recombinant strain featuring the highest recorded space-time, and yield, therefore making this strain a potential industrial tool for biotechnological rhamnolipid synthesis.

Burkholderia kururiensis KP23(T), a trichloroethylene-degrading, nitrogen-fixing and plant growth-promoting bacterium, has been reported to produce rhamnolipids, and when genetically engineered with two biosynthetic enzymes from *P. aeruginosa* RhlA and RhlB, rhamnolipid production increased by sixfold compared to wild-type strain. Rhamnolipids produced by the engineered strains were mainly mono-RL as opposed to wild-type strains *B. kururiensis* and *P. aeruginosa* that predominantly produce di-RLs. This organism is a promising biosurfactant producer with potential environmental and biotechnological application especially due to its nonpathogenicity and compatibility with metabolic engineering procedures (Tavares et al. 2013).

5 Conclusion

The development of biosurfactant production in nonpathogenic organisms is a current challenge that is receiving increased attention in order to avoid pathogenicity and complex metabolic regulations. Most biosurfactant-producing organisms are pathogenic and difficult to handle in large-scale industrial processes. Pathogenicity of biosurfactant producers is key factor to prevent large-scale production; therefore, the nonpathogenic eco-friendly organisms need to be explored further, and a library of collections is important for the future development in this area of study.

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Eco-Friendly Technologies for Heavy Metal Remediation: Pragmatic Approaches

Hemambika Balakrishnan and Rajeshkannan Velu

Abstract

Heavy metal contamination is a universal problem that disrupts the environment as a consequence of several anthropogenic activities. This chapter provides a review on the remediation technologies of heavy metal contamination. The modern remediation techniques of heavy metal from the contaminated soil and water are expensive and environmentally destructive. Unlike organic compounds, metals cannot degrade, and so efficient cleanup involves their immobilization to reduce or remove toxicity. The use of plants and associated microorganisms are gaining more attention to remove, immobile or degrade the environmental destructive contaminants. Phytoremediation is an emerging technology for cleaning up contaminated sites, which is cost effective, and has aesthetic advantages and long-term applicability. Furthermore, the metal-resistant bacteria are reported to play an important role in phytoremediation for successful survival and growth of plants. Moreover, the metal-resistant bacteria are reported to promote plant growth by various mechanisms such as nitrogen fixation, solubilization of minerals, production of phytohormones and siderophores, and utilization of 1-aminocyclopropane-1-carboxylic acid as a sole N source and transformation of nutrient elements. A brief review on phytoremediation of heavy metals and its effect on plants has been compiled to provide a wide applicability of phytoremediation.

Keywords

Heavy metals • Phytoremediation • Nitrogen fixation • Phytohormones • Siderophores

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1 Introduction

Industrial pollution is the most solemn problem to the environment that required main concern. A wide variety of chemicals and toxic wastes such as heavy metals and persistent organic pollutants, including pesticides, have been detected in different biota such as soil, water, and air (Turgut 2003). Among the pollutants, heavy metals pose a critical concern to human health and the environment due to their high occurrence as a contaminant, low solubility in biota, and the classification of several heavy metals as carcinogenic and mutagenic (Alloway 1995). Moreover, the metals cannot be degraded to harmless products and hence persist in the environment indefinitely. As a result, many different remediation methods have been tried to address the rising number of heavy metal-contaminated sites. Conventional cleanup technologies are expensive and feasible only for small but heavily polluted sites where rapid and complete decontamination is required. In addition, some methods, such as soil washing, electrokinetic remediation, and vitrification, can pose an adverse effect on biological activity, soil structure and fertility and acquire significant engineering costs (Pulford and Watson 2003). Therefore, sustainable on-site techniques for remediation of heavy metal-contaminated sites need to be developed.

2 Bioremediation Process

Various bioremediation techniques are employed for remediation of heavy metal-contaminated sites depending on the degree of saturation and aeration of an area. In situ techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance, whereas ex situ techniques are applied to the site which has been removed via soil excavation or water pumping.

2.1 In Situ Bioremediation

In general, in situ techniques are the most desirable options due to lower cost and fewer disturbances because they provide the treatment in place avoiding excavation and transport of contaminants. In situ treatment is certainly limited by the depth of the soil that can be effectively treated. The most important in situ land treatments are bioventing, biosparging, and bioaugmentation (Jadia and Fulekar 2009). Bioventing is the most common in situ treatment that involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. In situ biodegradation involves supplying oxygen and nutrients by circulating aqueous solutions through contaminated soils to stimulate naturally occurring bacteria to degrade organic contaminants. Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Bioaugmentation involves the addition of microorganisms indigenous or exogenous to the contaminated sites to enhance the degradation of the contaminants.

2.2 Ex Situ Bioremediation

The ex situ techniques involve the excavation or removal of contaminated soil from the ground. The most important ex situ treatments are landfarming, composting, biopiles, and bioreactors. Landfarming is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The main purpose is to stimulate indigenous biodegradative microorganisms and facilitate the aerobic degradation of contaminants. Biopiles are a hybrid of landfarming and composting, typically used for treatment of surface contamination with petroleum hydrocarbons (Fahnestock et al. 1998). It is a refined version of landfarming that tends to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic and anaerobic microorganisms. Bioreactors, slurry reactors, or aqueous reactors are used for ex situ treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the processing of contaminated solid material (soil, sediment, sludge) or water through an engineered containment system. In general, the rate and extent of biodegradation are greater in a bioreactor system than in in situ or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable.

3 Plant-Assisted Bioremediation

Phytoremediation for environmental cleanup is a promising tool, which is cost-effective, and has aesthetic compensation and long-term applicability. Phytoremediation is defined as the use of plants for removal of environmental pollutants or detoxification to make them harmless. Macek et al. (2000) have reported that the advantages of phytoremediation, which is due to its eco-friendly approach, public acceptance, and capability of degradation of diverse range of contaminants. Plants function in phytoremediation in two ways, the major one being facilitation of favorable conditions for microbial degradation, specifically by plant root-colonizing microbes, and the second aspect is plant root itself, providing a simple and inexpensive means of accessing contaminants existing in subsurface soils and water. Upon absorption of contaminants into the root, the chemical migrates through the root xylem and via the sap and eventually reaches the leaves (Suresh and Ravishankar 2004). Thus, phytoremediation shows potential for accumulating, immobilizing, and transforming a low level of persistent contaminants. Based on the fate of contaminants, phytoremediation techniques can be categorized broadly into five types such as phytoextraction, phytostabilization, phytodegradation, phytovolatilization, and rhizofiltration (Table 1).

3.1 Phytoextraction or Phytoaccumulation

This is a process used by the plants to accumulate contaminants into the harvestable parts of roots and aboveground shoots (Kumar et al. 1995; Chaney et al. 1997). This technique saves tremendous remediation cost by accumulating low levels of contaminants from a widespread area. Unlike the degradation mechanisms, this process produces a mass of plants and contaminants (usually metals) that can be then harvested and incinerated and the ash related to a confined area or the heavy metals are extracted from it.

Table 1 Categories of phytoremediation processes and mechanisms of contaminant removal

Sl. no.	Process	Plant mechanism	Contaminant	Substrate
1	Phytoextraction	Hyperaccumulation, uptake and concentration of metals via soils, direct uptake into the plant tissue with subsequent removal of the plants	Inorganics	Soils
2	Phytostabilization	Complexation; root exudates cause metal to precipitate soils, groundwater, and mine tailing and become less available	Inorganics	Soils, groundwater, mine tailing
3	Phytodegradation	Degradation in plants, enhances microbial degradation in rhizosphere	Organics	Soils, groundwater within rhizosphere
4	Phytovolatilization	Volatilization by leaves; plants evapotranspirate selenium, mercury, and volatile hydrocarbons	Organics/inorganics	Soils and groundwater
5	Rhizofiltration	Rhizosphere accumulation, uptake of metals into plant roots	Organics/inorganics	Surface water and water pumped

The metal concentrations in their dried foliage for a specific hyperaccumulator are as follows: 100 mg kg^{-1} of Cd, Se, and Tl; 300 mg kg^{-1} of Co, Cu, and Cr; 1,000 mg kg^{-1} of Ni, Pb, and As; 3,000 mg kg^{-1} of Zn; and 10,000 mg kg^{-1} of Mn when grown in its natural habitat (van der Ent et al. 2013). Apart from metal tolerance, hyperaccumulation is thought to benefit the plant by means of allelopathy, defense against herbivores, or general pathogen resistance (Boyd and Jaffré 2001; Davis et al. 2001). In the case of phytomining, the use of native flora (including local populations of hyperaccumulators) with limited agronomic practices (extensive phytoextraction) could be an alternative to intensively managed crops. In situ phytoextraction of Ni by a native population of *Alyssum murale* on an ultramafic site (Albania) has been reported by Bani et al. (2007). Lasat et al. (1998) conducted a field study to investigate the potential of three plant species for phytoremediation of a ^{137}Cs-contaminated site. Approximately 40-fold more ^{137}Cs was removed from the contaminated soil in shoots of redroot pigweed than in those Indian mustard and tepary bean. Among the plants, *Urtica dioica* found to be very effective due to its higher uptake capacity for Cr. *Zea mays* has been showed that high tolerance toward Cr with negligible concentration in leaves. Due to its higher Cr uptake and low biomass production of *U. dioica*, commonly known as "stinging nettle," it can be considered as the right plant for remediation of Cr-contaminated sites (Shams et al. 2010).

The lack of success of phytoremediation is largely related to the small biomass of most true hyperaccumulator plants or to metal accumulation by high-biomass (crop) plants being too low. For example, while contaminant mixtures appear to be the imperative rather than the exception at polluted sites, metal tolerance, as well as an efficient metal accumulation by a given plant species, is typically restricted to one or few elements. Moreover, high metal uptake rates in plants as required for phytoextraction can only be achieved if the metal activity in the rhizosphere soil solution is sustained by rapid resupply from the solid phase (Fitz et al. 2003; Lehto et al. 2006). The most studied approach is chelant-assisted phytoextraction using ethylene diamine tetraacetic acid (EDTA) and other artificial chelants (Wang et al. 2006).

3.2 Phytostabilization

In this technique, plants reduce the mobility and migration of contaminated soil. Leachable constituents are adsorbed and bound into the plant structure so that they form a stable mass of plant from which the contaminants will not reenter the environment. Most of the organic chemical contaminants are lipophilic and are attracted to the hydrophobic surfaces on organic matter, such as humus and plant cell wall components or soil particles (Rufyikiri et al. 2004; Shahandeh and Hossner 2002). Also, plants are used to reduce the bioavailability of environmental pollutants. Inoculation with metal-resistant plant growth-promoting bacteria (PGPB) can support the establishment and improve vitality of the phytostabilized crops, and detoxification mechanisms in the rhizosphere may be enhanced by inoculation with microbial associates. Some plants and microorganisms are able to precipitate metal compounds in the rhizosphere (Cotter-Howells and Caporn 1996) and may provide an effective means to reduce metal toxicity as well as metal mobility which is termed as phytoimmobilization (Cotter-Howells et al. 1999). The design of phytostabilization systems relates to combining different approaches to ameliorate multiple constraints (i.e., nutrient and water deficiency, toxicity due to mixed contamination) and to control their efficiency in field conditions (Roy et al. 2007).

3.3 Phytodegradation or Rhizodegradation

The breakdown of contaminants is through the activity existing in the rhizosphere due to the presence of proteins and enzymes produced by the plants or by soil organisms such as bacteria, yeast, and fungi. Rhizodegradation is a symbiotic relationship where the plants provide nutrients necessary for the microbes to thrive, while microbes provide an enhanced healthy soil environment. Contaminated soils that have undergone prolonged periods of ageing generally appear to be much less responsive to rhizodegradation than fresh soil (Chiapusio et al. 2007; Child et al. 2007). Characterizing root exudation in terms of chemical composition and quantity and investigation of utilization pattern by microbial strains competent to degrade the pollutants are important requirements for this purpose. In long-term field-contaminated soil, enhancement of bioavailability appears to be the key of successful biodegradation.

3.4 Phytovolatilization

It involves the use of plants to take up contaminants from the soil, transforming them into volatile forms and transpiring them into the atmosphere. Some metal(loid)s like arsenic (As), mercury (Hg), and selenium (Se) may exist as gaseous species in the environment. The contaminant available in the water taken up by the plant passes through the plant or is modified by the plant and is released to the atmosphere (evaporates or vaporizes). Some naturally occurring or genetically modified plants, like *Brassica juncea* (Indian mustard) and *Arabidopsis thaliana*, are reported to possess capability to absorb heavy metals and convert them to gaseous species within the plant and subsequently release them into the atmosphere (Ghosh and Singh 2005). Phytovolatilization has been used primarily for the removal of Hg, where in mercuric ion is transformed into the less toxic gaseous elemental Hg (Ghosh and Singh 2005). Some plants growing in high Se media, e.g., *A. thaliana* and *B. juncea*, produce volatile Se in the form of dimethylselenide and dimethyldiselenide (Bañuelos 2000).

3.5 Rhizofiltration

Rhizofiltration is a water remediation technique that involves the uptake of contaminants by plant roots, used to reduce contamination in natural wetlands and estuary areas. It is defined as the use of plants, both terrestrial and aquatic, to absorb, concentrate, and precipitate contaminants from polluted aqueous sources in their roots. It remediates metals like Pb, cadmium (Cd), Ni, Cu, Cr, vanadium (V), and radionuclides [uranium (U), cesium (Cs), strontium (Sr)]. The ideal plants should produce significant amounts of root biomass or root surface area that could accumulate and tolerate significant amounts of target metals, involve easy handling and a low maintenance cost, and have a minimum of secondary wastes that require disposal (Dushenkov and Kapulnik 2000). Sunflower, Indian mustard, tobacco, rye, spinach, and corn have been studied for their ability to remove Pb from water, with sunflower having the greatest ability. Indian mustard has a bioaccumulation coefficient of 563 for Pb and has also proven to be effective in removing a wide concentration range of Pb (4–500 mg/L) (Raskin and Ensley 2000).

4 Chelators in Phytoremediation

The chemical amendments such as synthetic organic chelates can enhance phytoremediation efficiency by increasing heavy metal bioavailability in soil thus enhancing plant uptake and translocation of metals from the roots to the aerial parts of host plants (Purakayastha et al. 2008). Among the chelates, EDTA was often found to be the most effective (Zaier et al. 2010). Huang et al. (1997) found that among different chelating agent, EDTA is more effective in the accumulation of Pb in corn and pea and also found that on increasing the concentration of EDTA, accumulation efficiency of Pb in the shoot of corn and pea was also increased. Therefore, the potential risks of use of EDTA or other chelators for phytoremediation should be thoroughly evaluated before taking steps toward further development and commercialization of this remediation technology. The use of chelates as soil amendments to increase the bioavailability of metals has raised some concern over the potentially increased mobility of the metal–chelate complex in the soil.

Several authors have emphasized the possibility of heavy metal groundwater contamination or other off-site migrations (Huang and Cunningham 1996). The toxicity of EDTA on soil bacteria, actinomycetes, and fungi relatively gives an indication of environmental stress inflicted also on microbial populations (Vestal and White 1989).

5 Bacteria in Phytoremediation

The success of the phytoremediation process whereby metals are effectively removed from soil is dependent on an adequate yield of plants and on the efficient transfer of metals from the roots of the plants into their shoots. Some plant species, such as *Thlaspi*, *Urtica*, *Chenopodium*, *Polygonum sachalase*, and *Alyssum*, growing in heavy metal-contaminated sites have been found with the ability to accumulate unusually high concentrations of heavy metals without impacting on their growth and development (Rajkumar et al. 2012). Such plants are termed as hyperaccumulators. However, most hyperaccumulators identified so far are not suitable for field phytoremediation applications due to their small biomass and slow growth (Puschenreiter et al. 2001). Moreover, the elevated metal levels are generally toxic to most plants, impairing their metabolism and reducing plant growth (Mohanty et al. 2005). These properties have an adverse impact on the potential for metal phytoremediation and restrict the employment of this technology. In this regard, interactions among metals, microbes, and plants have attracted attention because of the biotechnological potential of microorganisms for metal removal directly from polluted medium and the possible role on plant growth promotion in metal-contaminated soils.

There is a great deal with the rhizosphere bacteria that contribute to the metal extraction process, but the inherent mechanisms of this plant–microbe interaction are yet to be fully innovated. The rhizosphere of heavy metal-accumulating plants affords a niche for adapted metal-resistant microorganisms (Idris et al. 2004), and the mobility of heavy metals is higher in the rhizosphere of metal accumulators than in bulk soil, due to active mobilization by roots and microorganisms (Lasat et al. 1996). The plant growth-promoting hormones such as indole acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, siderophores, organic acids, or specific ligands produced by bacteria have been associated with enhanced growth and accumulation and mobilization of heavy metals under heavy metal exposure (Glick 2003; Hemambika and Rajesh Kannan 2012). Hence, among the rhizosphere microorganisms involved in plant interactions with the soil milieu, the PGPB deserve special attention (Glick 2003).

Further, ACC deaminase-producing bacteria play an important role in the alleviation of different types of stress in plants, including the effect of heavy metals (Glick et al. 1999). Moreover, different heavy metal tolerance mechanisms have also been discovered in various microbes: they involve exclusion, active removal, biosorption, precipitation, or bioaccumulation both in external and intracellular spaces (Hemambika et al. 2013). These processes can influence the solubility and the bioavailability of the metal to the plant, thus modifying the toxic effects of the metal. There is increasing evidence that besides climatic factors and soil properties, plant–microbe interactions determine the efficiency of metal phytoextraction. In some cases, rhizosphere bacteria enhance plant uptake of trace elements (Sheng and Xia 2006), whereas in other cases, they reduce or have no effect on the uptake (Madhaiyan et al. 2007). For example, Rajkumar and Freitas (2008) reported that the addition of *Pseudomonas jessenii* to the surface-sterilized root of *Ricinus communis* in autoclaved soil increased Zn concentrations in shoot tissues compared with non-inoculated controls.

In some plants, restriction of shoot translocation is believed to be the approach of metal tolerance for non-hyperaccumulators. However, the capacity to accumulate heavy metals in aboveground plant tissues represents a central point for the suitability of the plants for metal phytoextraction (Salt and Kramer 2000). The amount of metals accumulated in the aerial plant part may vary during the growing season as a consequence of the inherent growth dynamics of the plant, as well as in response to variations in the heavy metal levels and availability in the surrounding water and soil (Hardej and Ozimek 2002). *Pleurotus australis* showed a very high adsorption affinity for Cu, Cd, Ni, Pb, and Zn from aqueous solutions (Southichak et al. 2006). Interactions between the microbial community, species composition, soil/sediment characteristics, and hydrology play an important role in the types and rates of remediation reactions that occurred in the contaminated environment. Williams et al. (1999) mentioned that successful long-term phytoremediation must carefully consider the management implications of secondary succession to avoid ecological shifts away from the optimal plant community structure.

6 Harvesting and Recycling of Plant Biomass

The role of plants in the phytoremediation of heavy metals were considered to be a successful, low-cost, cleanup option to ameliorate the quality of contaminated soil (Gopal 2003). They not only assimilate pollutants directly into their tissues but also they act as catalysts for purification reactions by increasing the environment diversity in the root zone and promoting a variety of chemical and biochemical reactions that enhance purification (Jenssen et al. 1993). Vajpayee et al. (2001) demonstrated this concept following metal uptake tests with *Vallisneria spiralis*, a freshwater submerged, rooted wetland species that were tested for Cr accumulation in microcosms and were found to effectively remove Cr by adsorption and absorption into plant tissues, hence, concluded that *V. spiralis* may be suitable as Cr accumulators in constructed wetlands, where biomass could be safely harvested and disposed off. Harvested plant biomass may also be used in biogas production, resulting in a safe and advantageous disposal.

Phytoremediation involves repeated cropping of plants in contaminated soil, until the metal concentration drops to acceptable level. The ability of the plants to account for the decrease in soil metal concentrations as a function of metal uptake and biomass production plays an important role in achieving regulatory acceptance. Hypothetically, metal removal can be accounted by determining metal concentration in plant, multiplied by the biomass produced, and comparing it with the reduction in soil metal concentrations. There are many factors that make it challenging in the field. One of the constraints for commercial implementation of phytoremediation has been the disposal of contaminated plant material. After cropping, the plant is removed from the contaminated site that leads to accumulation of huge quantity of hazardous biomass. This hazardous biomass should be stored or disposed properly so that it does not pose any risk to the environment. Biomass is nothing but stored solar energy in plant mass, which is also termed as materials having combustible organic matter. Biomass contains carbon, hydrogen, and oxygen and is known as oxygenated hydrocarbons. The main constituents of any biomass material are lignin, hemicellulose, cellulose, mineral matter, and ash. It possesses high moisture and volatile matter constituents, low bulk density, and calorific value. The percentage of these components varies from species to species. The dry weight of *B. juncea* for induced phytoextraction of Pb amounts to 6 tonnes per hectare with 10,000–15,000 mg/kg of metal in dry weight (Blaylock et al. 1997). Handling of huge quantity of this type of waste is a problem and hence need volume reduction (Blaylock and Huang 2000).

Plant-associated microbes play a very important role in heavy metal removal from industrial waste-contaminated soils. However, uptake of metals by microbes and host plants is only a temporary removal process, and the harvesting and removal of biomass are an essential step for effective removal of heavy metals from contaminated environment. Therefore, this aspect needs separate attention. Weis and Weis (2004) mentioned that senescent plant tissues may be sources of metals released through leaching or can be sinks for metals through litter adsorption or microbial immobilization. They also stated that the extent of uptake and the distribution of metals within plants have important effects on the resistance time of metals in plants and in contaminated area and the potential release of metals. The biomass collection problems can be illustrated by the concerns raised by Khan et al. (2000) and concerns regarding contaminated plant biomass following accumulation.

Composting and compaction have been proposed as postharvest biomass treatment (Garbisu and Alkorta 2001). Studies carried out by Hetland et al. (2001) have showed that composting can significantly reduce the volume of harvested biomass. However, metal-contaminated plant biomass would still require treatment prior to disposal. Total dry weight loss of contaminated plant biomass by compaction is advantageous, as it will lower the cost of transportation to a hazardous waste disposal facility.

One of the conventional and promising routes to utilize biomass produced by phytoremediation in an integrated manner is through thermochemical conversion process. If phytoextraction could be combined with biomass generation and its commercial utilization as an energy source, then it can be turned into a profit-making operation, and the remaining ash can be used as bio-ore (Brooks et al. 1998); this is also the basic principle of phytomining. Nicks and Chambers (1994) also reported a second potential use for hyperaccumulator plants for economic gain in the mining industry. This operation, termed phytomining, includes the generation of revenue by extracting saleable heavy metals produced by the plant biomass ash, also known as bio-ore.

Phytomining is the recovery process of accumulated trace metals, may be an additional benefit of phytoremediation. However, concerns about plant matter getting into the food chain by direct consumption or by decomposition pathways include considerable ecological and human health problems (Khan et al. 2000). Also, the recovery of metals is very costly. Hence, harvesting and disposing of plant biomass are essential to prevent recycling of accumulated metals when plants are decomposed. Hetland et al. (2001) presented three approaches for processing of bioaccumulating plant tissues. Biomass recycling is a foremost concern to address because the environmental factors or the livestock may remove fresh biomass prior to harvesting. The transfer of heavy metals from the contaminated area into plant matter may help to resolve biomass-recycling concerns.

Combustion and gasification are the most important sub-routes for organized generation of electrical and thermal energy. Recovery of this energy from biomass by burning or gasification could help make phytoextraction more cost-effective. Thermochemical energy conversion best suits the phytoextraction biomass residue because it cannot be utilized in any other way as fodder and fertilizers. Combustion is a crude method of burning the biomass, but it should be under controlled conditions, whereby volume is reduced to 2–5 % and the ash can be disposed properly (Bridgwater et al. 1999). It is not encouraged to burn the metal bearing hazardous waste in the open, as the gases and particulates released in the environment may be detrimental; only the volume is reduced and the heat produced in the process is wasted. Gasification is the process through which biomass material can be exposed to series of chemical changes to yield clean and combustive gas at high thermal efficiencies. This mixture of gases is called as pyro-gas that can be combusted for generating thermal and electrical energy.

Bridgewater et al. (1999) have reported that pyrolysis is a novel method of municipal waste treatment that might also be used for contaminated plant material. Pyrolysis decomposes material under anaerobic conditions, and there is no emission to the air. The final products are pyrolytic fluid oil and coke; heavy metals will remain in the coke, which could be used in smelter. Koppolu et al. (2003) reported that 99 % of the metal recovered in the product stream was concentrated in the char formed by pyrolyzing the synthetic hyperaccumulator biomass used in the pilot scale reactor. Helson et al. (1997) conducted low temperature pyrolysis experiments with Cr-, Cu-, and As-treated wood, and it was concluded that most of the metal was retained in the pyrolysis residue. Influence of metal ions on the pyrolysis of wood has been studied extensively by many authors (Pan and Richards 1990; Richards and Zheng 1991; Mohan et al. 2006, 2014).

7 Advantages and Disadvantages of Phytoremediation

Phytoremediation is well processing methods were used at large contaminated sites, where other methods of remediation are not cost-effective or practicable; at sites with a low concentration of contaminants where only polish treatment is required over long periods of time and in combination with other technologies where vegetation is used as a final cap and closure of the site. There are some limitations to the technology which include long duration of time for remediation, potential contamination of the vegetation, and food chain difficulty in establishing and maintaining vegetation at some sites with high toxic levels.

8 Integrated Approaches

The complexity and heterogeneity of sites often polluted with multiple metals, metalloids, and organic compounds require the design of integrated phytoremediation systems that combine different processes and approaches. Co-cropping of different species may enhance the overall capabilities of a phytoremediation system to explore the contaminated soil volume, address different pollutants, and support differential microbial consortia in their rhizospheres. Shared rhizospheres may be designed to optimize the nutritional status, e.g., by combining plants that support N_2-fixation and P-solubilizing microorganisms. Co-cropping could be also used to modify the bioavailability of pollutants with the plants (Roy et al. 2007).

9 Conclusion and Future Prospects

The success of a phytoremediation technique is largely dependent on the continuous metal availability that concerns the plants. It appears attractive because in contrast to most other remediation technologies, it is not invasive and, in principle, delivers intact, biologically active soil. There is a need to enhance research efforts on this emerging technology. Phytoremediation research should focus on identifying indigenous hyperaccumulators that are adapted to the local climate and soil conditions. Since phytoremediation is a slow process, biotechnological as well as conventional hybridization techniques should be used to develop more efficient metal hyperaccumulator plant species having increasing pollutant tolerance, root and shoot biomass, root architecture and morphology, pollutant uptake properties, and degradation capabilities for contaminants. Additional strategies should include proper management of the soil, e.g., via fertilization or chelant addition to increase pollutant bioavailability, and of the phytoremediation crops, e.g., via optimization of coppicing, harvest cycles, and development of mixed cropping systems.

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Phytoextraction of Trace Metals: Principles and Applications

Tiziana Centofanti

1 Introduction

Trace elements (TEs) occur at minor concentration (>1 g kg^{-1}) in the organisms, and some are essential nutrients (Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, B, and Cl) for animals and plants. As a consequence of human activities such as industrial production, mining, transport, and agriculture, they are released in the environment at high concentrations. TEs can accumulate over time under specific environmental conditions, thus becoming environmental contaminants (Cs, Cr, W, U, Cd, Hg, Tl, Pb, Sn, As, Sb, Se). The environmental risk of TEs is associated with the mobility and bioavailability of the metals more than their total concentration. When they become environmentally mobile and move between media (i.e. soil to water), they can enter the food chain by being taken up by plants and animals. TEs cannot be degraded or broken down and at high concentration are toxic to organisms and tend to bioaccumulate in the environment. For example, selenium (Se) is a naturally occurring element with a wide distribution in almost all parent materials on Earth. At low concentration, Se is an essential nutrient but at high concentration is toxic. In the western side of the San Joaquin Valley in California, soils contain significant quantities of soluble mineral salts and trace elements such as Se and boron (B) that have been leached into shallow groundwater and/or drainage waters because of irrigation practices. Soluble Se bioaccumulated in the avian food chain and resulted in an environmental disaster with high mortality and reproduction failure of migratory birds (Letey et al. 2002; Ohlendorf et al. 1986).

According to the German Advisory Council on Global Change, 22 million hectares of land are contaminated with TEs worldwide (GACGC 1994). Specifically, a comprehensive inventory of global soil contamination is lacking (www.globalsoilweek.org). For example, at the European level, it is unclear what threshold values should be used to classify a soil as polluted and with regard to TEs at which locations can high natural background values be expected (Morvan et al. 2008).

For the government (i.e. USA and EU) to act, usually the contamination must be severe enough to cause barren soils, limit crop production, and cause groundwater contamination and unsafe living conditions. Lack of global inventory of soil contamination and of consequent public awareness of the health risks is one of the reasons why governments fail to require and fund remediation in areas that are of low economic value such as marginal agricultural land, former mining areas, landfills, and postindustrial sites. Knowledge of

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the extent and the location of the contaminated areas and assessment of the environmental risk associated with the contamination would trigger reaction and concerns in the local community near or close the contaminated area. Ecosystem disruption is not enough to trigger action by the government. In the majority of cases when contamination is 'mild' and does not cause evident unsafe living conditions, the economic and social responsibility of cleaning up the site or setting it aside from food production is in the hands of the landowner.

Most commercial soil remediation strategies rely on the use of engineered methods such as soil excavation (dig and haul), pump-and-treat systems, soil washing, leaching, and soil capping. These methods are effective and are usually applied when the contaminated area has commercial value (i.e. growth of urban areas) and the soil needs to be remediated in short time. However, these methods are prohibitively expensive and destructive of the soil ecology and fertility. They also generate high amounts of waste that needs to be disposed of (Conesa et al. 2012).

Phytotechnologies represent a green alternative to conventional remediation methods because they are based on the use of solar-driven biological processes to remediate or reduce the risk of contamination. Phytotechnologies are low cost and gain wide public acceptance because of the environmental benefit they provide to the remediated areas, i.e. revegetation, decreased formation of soil dust, reduced soil erosion, carbon sequestration, etc. They comprise a number of different methods that aim at removing, extracting, transforming, and immobilising contaminants using plants and their associated root microorganism (Pilon-Smits 2005).

A number of authors have described in detail the different phytoremediation technologies and their advantages and disadvantages and their applications (Chaney et al. 2007; Dickinson et al. 2009; Pilon-Smits 2005). In this chapter, we focus on cost-effective phytoextraction and on the economic viability and environmental benefits of phytoextraction as successful commercial phytotechnology.

2 Limits of Phytoextraction

Phytoextraction is the removal of TEs from the soil by growing plants that have the ability to take up TEs in their above-ground biomass at high concentrations. Harvest of the TE-rich biomass and multiyear growth cycles of the plants may allow the removal of the TEs from the soil to a concentration level acceptable by the environmental regulatory authority. The removed biomass that has no value as bio-ore is usually incinerated, composted, or digested to reduce the volume and disposed in landfills or in hazardous waste landfills. Chaney et al. (2010) note that disposal of biomass represent only a disposal cost rather than a problem in most cases except for radionuclides.

The development of phytoextraction has begun a few decades ago with the discovery of hyperaccumulator plants by pioneering studies by Robert Brooks (Brooks et al. 1977), Alan Baker (1981), and Rufus Chaney (1983). TE concentrations in the shoots of hyperaccumulator are about 100–1,000 times higher than that found in normal plants under most circumstances. Specifically, the concentration values (in their dried foliage) to define a hyperaccumulator are as follows: 100 mg kg^{-1} of Cd, Se, and Tl; 300 mg kg^{-1} of Co, Cu, and Cr; 1,000 mg kg^{-1} of Ni, Pb, and As; 3,000 mg kg^{-1} of Zn; and 10,000 mg kg^{-1} of Mn when grown in its natural habitat (van der Ent et al. 2013). Based on this criteria, more than 500 plant taxa have been cited as 'hyperaccumulators' of one or more elements including As, Co, Cd, Cu, Mn, Ni, Pb, Se, Tl, and Zn. At present, the approximate number of hyperaccumulators for various elements is as follows: Ni (450), Cu (32), Co (30), Se (20), Pb (14), Zn (12), Mn (12), As (5), Cd (2), and Tl (2) (van der Ent et al. 2013). Nearly 25 % of hyperaccumulators belong to the family of Brassicaceae and the genera *Thlaspi* and *Alyssum*.

The benefit and adaptive advantage of hyperaccumulators have not yet been explained, but a variety of hypotheses have been proposed. The most popular one is the 'elemental defence' hypothesis (Boyd 2007) which suggests that the high amounts of TEs in the shoots of hyperaccumulator plants render them less palatable to pathogen and herbivores, thus reducing the possibility of their attacks and stimulating a defence against them. Despite the numerous studies supporting this hypothesis, more information is required since only a few taxa and a limited number of TEs have been analysed.

One of the key factors for the success of phytoextraction is the high uptake of TEs in the above-ground biomass, and that is why hyperaccumulators have been widely studied for the commercial development of phytoextraction technologies. The ability of hyperaccumulators to take up high amount of TEs depends on two unique traits, such as the constitutive up-regulation of transmembrane metal transporter, which confers faster and effective root-to-shoot translocation of TEs, and hypertolerance, which is mostly dependent on effective detoxification and storage of TEs in leaf cell vacuoles. Another very important characteristic of hyperaccumulators relative to normal crops is a greater ability to take up TEs from the soil.

It has been shown that hyperaccumulators absorb metals from the same labile pool in soils as normal plant species; however, crop plants cannot absorb high amounts of TEs to support phytoextraction. Despite the recent advancements in understanding the physiological mechanisms of metal uptake and translocation to shoot (Milner and Kochian 2008), no mechanisms are yet known where hyperaccumulator plants can attack the non-labile pool of metals in soils. Centofanti et al. (2012) investigated whether the Ni hyperaccumulator *Alyssum corsicum* possess distinct extraction mechanisms for different Ni species present in soils, as they have different solubility and potential bioavailability to roots. Their study showed that Ni uptake is related to Ni solubility and plant transpiration rate. The authors also suggested that Ni uptake is driven by convection, which depends on the initial concentration of Ni in solution and the plant transpiration rate. Metals enter the roots via uptake of the soil solution, which is then transferred to the stems and leaves and lost via transpiration. High metal concentration in the roots can result from plant water uptake inducing metal migration via mass flow (Zhao et al. 2000). Hyperaccumulators have the ability to translocate the absorbed metals from the roots to the shoot and store them in the leaf cell vacuoles (Broadhurst et al. 2004, 2009; Tappero et al. 2007).

The ability of plants, and of trees in particular, to pump large amount of water and solutes has been used to decrease the downward movement of solutes and leaching into the groundwater and to stabilise and break down contaminants in soil and groundwater. A successful example of boron (B) phytoextraction has been reported by Robinson et al. (2003a, 2007) where high water-use poplar trees were used to evapotranspire water, control leaching, and remove B from the site by coppicing the trees that accumulated significant amount of B in their leaves. Fast-growing and metal-resistant trees (i.e. *Salix* spp.) have two advantages relative to hyperaccumulator plants: (1) extracting more metals from the soil because of their large biomass in both above and below ground (Pulford and Watson 2003) and (2) stabilising the metals in the soil and reducing soil erosion by wind and water.

The low biomass production is the major limitation to the commercial development of phytoextraction using hyperaccumulator plants. Most hyperaccumulator species have a small size and usually small leaf area, thus producing little biomass compared to crop plant and trees. Robinson et al. (2003b) suggested a model to calculate the time needed for phytoextraction to lower the contaminant concentration in soil to the level required by environmental regulation. Phytoextraction is a time-consuming process because it is dependent on biomass production and ability of the plant to take up metals that is a function of root exposure to bioavailable TEs. Low biomass production results in low evapotranspiration, which affects the uptake and translocation of metals from the soil solution. In addition, the distribution of TEs in the soil profile is heterogeneous, and the plant roots might not have access to the TE ‘hot spots’. Furthermore, hyperaccumulator are metal specific and can only extract high concentrations of one contaminant. Therefore, remediation of a site polluted with more than one TE might require sequential phytoextraction with different species, a process that will lengthen the time needed to clean up the site. On average, the time required for phytoextraction to clean even a moderately contaminated soil is in the order of decades.

Induced phytoextraction to increase metal availability has been studied in the past. It consists in addition of solubilising metal chelators (i.e. EDTA, ethylenediaminetetraacetate) to the soil to increase the mobility and allowing the metals to be taken up more easily by the plant. However, induced phytoextraction has no application in situ because it poses an environmental risk through metal leaching to groundwater and it is cost prohibitive (Chaney et al. 2007).

A more promising approach to increase the biomass and obtain hyperaccumulation is being studied where all genes needed for hyperaccumulation and hypertolerance are cloned and expressed in a high biomass plant (Cherian and Oliveira 2005; Clemens et al. 2002; Dhankher et al. 2012; Rugh et al. 1998). However, the development of a high biomass bioengineered metal hypertolerant and hyperaccumulator plants is still in its infancy. Hyperaccumulator traits are expressed in several genes, and the engineering of transgenic plants might become impractical beyond a certain number of genes (Krämer 2010).

Phytoextraction is a low-cost environmentally friendly technology, but the fact that it is 'time consuming' (Conesa et al. 2012) adds additional unpredictable costs and makes it less appealing relative to engineering methods or nonaction. One of the major drawbacks of phytoextraction that hinders the commercial development of the technology is the cost efficiency. Growing plants for cleanup of a contaminated soil is costly, and the cost of production (fertilisers, irrigation water, harvest machines, etc.) adds up to the cost of biomass disposal. Farmers and landowners may desire soil cleanup, but the decision to take action is dependent upon the economics of their farm operations.

The importance of agricultural management practices for producing high yield for phytoextraction crops has often been overlooked. Chaney et al. (2007) describe the agronomy of phytoextraction and point out the importance of practices such as fertilisation, pH optimising, weed control, and scheduling of harvest as critical field management practices for the success of phytoextraction. However, field management operation and agricultural practices need to be included in the cost analysis of phytoextraction and in models to estimate value and cost of phytoextraction products (Robinson et al. 2003b). When the phytoextraction is combined with a profit-making operation, then the time constraint may become less important. In addition, when the phytoextraction product (biomass) has commercial value (timber, bioenergy, fertiliser, food supplement, etc.), it can be sold to offset the cost of production and farm practices operation.

There are only few successful demonstrations of the possibility of using phytoextraction in field sites and production of secondary products that offset the costs of production. Perhaps the most economically viable example is the use of Se-enriched crops (i.e. *Opuntia ficus-indica* (cactus pear) and *Brassica oleracea* L. (broccoli)) (Bañuelos et al. 2012) for human consumption and production of forages (*Brassica napus* L., canola) with enough Se to replace Se supplements normally added to livestock feed (Bañuelos 2006). The oil and seed meal extracted from *Brassica* seeds grown in Se-rich soils of the San Joaquin Valley in Central California have been used as source of biofuels, green fertilisers, and bioherbicide (Bañuelos 2009; Bañuelos and Hanson 2010).

Two other important ways to combine phytoextraction with production of secondary products, such as biomass for bioenergy and recovery of metals from the plant as bio-ore, are described in the following sections.

3 Phytoextraction and Bioenergy Production

Biofuels are renewable fuels derived from biological feedstock and are largely carbon neutral because the CO_2 released during biofuels combustion is offset by carbon fixation during plant growth. Biofuels are considered as a key to reducing reliance on foreign oil, lowering greenhouse gas emissions, and meeting rural development goals by developing local and sustainable energy sources. However, the political and public support for biofuels has been undermined by concerns related to food security because the conversion of croplands to produce biofuels may cause food shortages and associated increase in food prices (Koh and Ghazoul 2008).

Utilisation of poor-quality soils and contaminated land can extend the area available to grow energy crops, and it can avoid competition between energy crops and food products. Poplar (*Populus* spp.) and willow (*Salix* spp.) have been demonstrated to be the most successful tree crops that can be grown on contaminated land for biomass production and phytoextraction of TEs (Pulford and Watson 2003). They are fast to propagate, have many and deep roots, achieve high annual biomass production, take up large quantities of water, and generally possess high tolerance to trace metals (Cd, Cu, Zn, Pb) (Granel et al. 2002; Hu et al. 2013; Maxted et al. 2007; Meers et al. 2007; Mirck et al. 2005; Vervaeke et al. 2003). *Salix* and *Populus* spp. have an effective nutrient uptake and high evapotranspiration rate and a pronounced clone-specific capacity for heavy metal uptake. Success of *Salix* spp. (willow) as phytoextracting plants depends on its biomass production, metal accumulation capacity, and the site of metal accumulation in the plant. Willow is usually grown in short-rotation coppice (SRC) systems because plants have the ability to resprout after harvest. This characteristic makes willow very suitable to phytoextraction because the frequency and number of harvests will trigger higher metal removal. The estimated economic lifespan of a short-rotation willow coppice stand is 20–25 years, with 6–7 harvests (the time frame from planting to first harvest is typically 4 years).

Research on the environmental sustainability of willow production began in the mid-1980s by various groups in the USA and Europe (Gomes 2012; Rowe et al. 2009; Šyc et al. 2012; Volk et al. 2006). The willow cropping system utilises agricultural practices that are familiar to farmers, and after establishment, it is a relatively low-input crop with winter harvests, thus having a limiting effect on other farming operations. Willow biomass production systems involve intensive site preparation to control weeds, double-row mechanical planting of high density (15,300 plants ha^{-1}), nitrogen inputs at the beginning of each rotation, and 3–4-year rotations. It has been demonstrated that the use of cover crops during the establishment phase of willow plantations and mechanical control of the cover crops (such as rolling, undercutting, or partial rototilling) will reduce the risk of erosion during tree establishment and will allow tree plantations on sloping farmland.

A common critique to the sustainability of willow SRC systems is the creation of ‘biological deserts’ across the landscape due to the monoculture of willow. However, long-term research on the above- and below-ground biodiversity in willow plantations has shown positive effects on avian biodiversity comparable to natural habitats including shrubland and successional habitats (abandoned fields, second-growth forests, regenerating clear-cuts) (Volk et al. 2006). Šyc et al. (2012) have suggested intercropping of fast-growing species such as willow and poplar with hyperaccumulators to increase the intake of metals for phytoremediation and to contribute to increases in biomass production and positive effects on biodiversity. The feasibility of intercropping hyperaccumulators with SRC needs to be studied in relation to impediment of mechanical operations for harvest and other agronomic practices (fertilisation and weed control).

It is generally expected that SRC will have higher water demand than arable crops due to the higher growth rates, high transpiration rates, and longer seasonal growth. In some European countries (i.e. UK), government guidelines require the plantation of SRC in areas where annual rainfall is at least 600 mm year^{-1} (Rowe at al. 2009). One environmental advantage of the high transpiration rate of willow is that the amount of water removed from the soil by the transpiration stream can decrease the downward flow through the soil and can reduce leaching losses. In addition, the perennial nature of SRC, their extensive root system, and the potential use of conservation tillage and cover crops will minimise nutrient outflow, strongly decrease the risk of soil erosion, and maintain good water quality (Abrahamson et al. 1998). Heller et al. (2003) carried out a life cycle assessment of willow plantation for bioenergy, and they showed that nitrogen fertilisation of willow accounts for the majority (37 %) of primary energy consumed over seven harvest rotations of willow biomass crops. The production of N fertilisers consumes large amount of non-renewable fossil fuel. The authors showed that substituting inorganic fertiliser with sewage sludge biosolids can increase the net energy ratio by more than 40 % (Heller et al. 2003). The net energy ratio for the production and conversion of short-rotation woody crops (SRWC) is 1:11, meaning that for every unit of non-renewable fossil fuel energy used to grow, harvest, and deliver SRWC, 11 units of usable energy are produced. In contrast, the net energy ratio for ethanol production from corn is 1:1.3 and for natural gas is 1:0.4. In essence, willow crops are large solar collectors that capture the sun's energy and store it in the woody biomass. In addition to the positive energy balances, willow crops can revitalise local economies and sustain rural development by diversifying farm crops and income.

Despite the numerous environmental and rural development benefits of SWRC, their use and cultivation have not been widely adopted in the USA and Europe. the main reason being the high cost of production ($\$2.60–3.00\ GJ^{-1}$ vs. $\$1.40–1.90\ GJ^{-1}$ for fossil fuels) due to high operating costs (low harvesting efficiency, high transportation costs), relatively low yield, and low energy conversion efficiency. One additional disadvantage of SRC biomass for bioenergy is the inability of SRC systems to continuously supply biomass throughout the year; the biomass is usually insufficient as a sole source of fuel for a combustion plant. Co-firing with coal and other fuel products could be envisaged if regulation is respected. To avoid air pollution, combustion of *Salix* wood grown on contaminated land should occur only in industrial and collective boilers equipped with efficient filters that trap the volatile particles containing the contaminant (i.e. Cd). The bottom ash can be used as basic mineral amendment or inorganic fertiliser. Co-firing a 100 MW power plant with 10 MW of willow biomass would require approximately 4,000 ha (10,000 acres) of biomass crop establishment, which corresponds to 1 % of the area in 80 km transport radius around the power plant (Abrahamson et al. 1998). The low energy conversion efficiency of SRWC is an additional problem that hinders the development of the technology on large scale. Alternative technologies including gasification and pyrolysis that can increase the overall conversion efficiency of biomass from SRWC are currently in various stages of research (Volk et al. 2006).

Lewandowski et al. (2006) quantified the economic value of combined cadmium remediation and biomass production by willow in a cadmium-contaminated case study area in the Rhine Valley (Germany). They concluded that the value of the phytoremediation function to farmers assessed by the substitution cost (alternative cost of soil cleanup by use of hyperaccumulator *T. caerulescens*) and hedonic price analysis (economic loss if the farmer cannot apply phytoremediation and has to set aside cadmium-contaminated land) delivers similar results and it is about 14,000 € ha^{-1} over a period of 20 years. However, farmers in the Rhine Valley were only willing to pay 0–1,500 € ha^{-1} mainly because they consider soil cleanup government's duty. In addition, farmers were negatively influenced by the fact that they considered contamination not being their fault, and none of the farmers interviewed tried to calculate the benefit of phytoremediation.

Ongoing research and development activities to promote commercialisation and development of biomass power are focused on improving socio-economic sustainability of SRC and promoting government actions that remove constraints and provide incentive for biomass fuel use. Biomass fuel can contribute to the economic longevity of local/rural coal-fired power plants and provide a carbon-neutral fuel for power producers to help them meet the goals of governmental climate change actions.

4 Phytomining

Commercial mining is usually performed on ores with high concentration of the target metal (for Ni, at least 30 g kg^{-1}) and are environmentally costly and energy- and capital-intensive practice of mining. Few ore bodies of this kind occur on the Earth's surface and are present in small localised areas, and some of these are becoming exhausted due to expanding economies and industrialisation.

Sub- or low-grade ores contain concentration of target metal below the content required to be economically extracted and smelted by conventional methods. Most of these ore bodies are associated with ultramafic deposits that generate serpentine soils after weathering of the ultramafic rocks. Serpentine soils are characterised by pH of 6–8; low Ca/Mg ratio and low levels of N, P, and K; and potentially toxic concentrations of Ni (Li et al. 2003a; Sheoran et al. 2009); they are not economic to mine and are unsuitable for agriculture due to high trace metal content (especially Ni). These deposits are scattered around the world and usually support a characteristic flora of endemic plants (Brooks 1987) that are able to tolerate and/or (hyper) accumulate the metals present in the serpentine soil. The use of plants to extract Ni and few other metals to produce a bio-ore is called phytomining. Dried plant material of hyperaccumulators grown on Ni-rich serpentine soil is reduced to an ash, and the metal is recovered using conventional metal refining methods such as acid dissolution or electrowinning.

The first field trial on Ni phytomining used a naturally occurring stand of *Streptanthus polygaloides* grown on serpentine soils in CA, and it extracted up to 100 Ni kg ha^{-1}, worth \$550 ha^{-1} at the prices of Ni in 1994 (Nicks and Chambers 1998). In a second phytomining field trial, *Alyssum bertolonii* (Robinson et al. 1997a) was used on a serpentine soil in Italy, and plants were fertilised with N, P, and K over a 2-year period. Fertilisation induced a threefold increase in dry biomass, indicating that agricultural practices similar to those applied to crops are important to increase yield in hyperaccumulator plants used for phytomining. Li et al. (2003b) demonstrated the feasibility of Ni recovery from the ash (bio-ore) of genetically improved *Alyssum* spp. Bio-ore contains higher Ni concentration (6–16 %) than normal Ni ores and are free of Mn, Fe, and Si oxides as in conventional ores (Robinson et al. 2009). Chaney et al. (2007) discuss the importance of fertilisation, pH optima, plant density, and development of improved cultivars for increasing shoot Ni concentration and yield of hyperaccumulator *Alyssum murale*. Weed management is also necessary when serpentine soils are fertilised, as other plants can compete with the hyperaccumulator grown for phytomining. In addition, in wet climate when soils are poorly drained, ridge tilling helped reduce adverse effect of heavy spring rainfall and consequent flooding on the survival and yield potential of *Alyssum* grown on a field trial in Canada (Chaney et al. 2007).

Further studies (Anderson et al. 1999; Harris et al. 2009; Keller 2004; Robinson et al. 1997b) have shown that other species could be grown for phytomining and that other metals could be extracted, such as thallium (Tl) and gold (Au). There are only a few Tl hyperaccumulators, namely, *Iberis intermedia* and *Biscutella laevigata*, from southern France (Leblanc et al. 1999) that can take up high level of Tl in dry matter, 0.4 mg kg^{-1} and 1.4 mg kg^{-1}, respectively. Tl is quite rare in nature, representing only 0.7 mg kg^{-1} in the Earth's crust. It is very toxic and is used in rat poison and for the control of ants. There is clearly potential for Tl phytomining if large areas are contaminated with Tl to obtain the advantage of large-scale operations. *I. intermedia* can produce 10 t ha^{-1}, as determined from field observation in France, and it should produce about 700 kg ha^{-1} of bio-ore containing 8 kg Tl (Anderson at al. 1999). Plants do not normally accumulate Au, and the metal must be made soluble before uptake can occur. Au is extracted by induced phytoextraction, which consists of using a chelating agent, usually thiocyanate, to make Au available to plant roots. It requires standard ore mining and grinding of the ore and then placing the ground ore over a plastic surface to avoid leaching of the cyanide and thiocyanide used to induce phytoextraction.

To be economically viable, phytomining should be able to produce about \$500 ha^{-1}, and most phytomining operation can gain additional revenue from incineration of biomass to generate electricity. The vicinity of the phytomining farm to the power plant is important to reduce transportation costs and to facilitate the secondary use of biomass for bioenergy production in addition to the recovery of the target metal. Furthermore, as noted by many authors, the lack of continuity of biomass supply from phytomining operation where harvest occurs once or twice a year is an impediment to the use of biomass for bioenergy unless other sources of biomass are available locally to provide material for the continuity of combustion operation throughout the year. One way to solve this problem is the co-firing with other sources of biomass as explained in Sect. 3.

A critique related to the environmental sustainability of phytomining addresses the efficiency of phytomining relative to conventional mining. Robinson et al. (2009) pointed out that phytomining requires a large area and more time to produce a ton of Ni than conventional mining (2.5 ha $year^{-1}$ vs. 22 m^3 in a few hours). In addition, Robinson et al. (2009) note that phytomining of surface serpentine soil will take from 3 to 18 crop cycles before the surface Ni is depleted, and the authors argue that the surface soil needs to be removed to continue phytomining of the deeper soil layers. The authors are also concerned with the introduction of a monoculture of possibly exotic species and disruption of serpentine ecosystem and native endemic flora.

Other authors (Anderson et al. 1999; Chaney et al. 2010; Harris et al. 2009; Sheoran et al. 2009) are supportive of phytomining and consider its environmental impact similar to that of commercial farming. Phytomining is considered to have a positive effect on soil erosion by the effect of plant roots relative to the open pit and the 'desert-like' landscape left after conventional mining operation has ceased and substantial site remediation is required at the end of the mine. Phytomining, instead, can improve the quality of the soil for post-mining operation over the duration of the phytomine. It is necessary, however, to study the potential environmental impacts of the phytomine during the planning phase and before the phytomining operation is set in place.

Hence, this technology requires a team of expert agronomists, ecologists, and soil scientists to carefully choose the proper species for phytoextraction of a particular metal. Various steps can be taken to reduce the ecological risks associated with monoculture and introduction of potential exotic species, for example, (1) analysis of phytoextraction potential of native vegetation and selection of the best performing native species for metal extraction efficiency, (2) adoption of principles of agro-ecology such as cultivation of combination of various native species, and (3) use of alternative fertilisers (sewage sludge biosolids) and bioherbicides.

Finally, conventional mining is not economically viable on low-grade ore, and hence a comparison of the efficiency between phytomining and conventional mining is not appropriate.

If phytomining proceeds beyond the theoretical and trail stage, the most likely scenario that can be envisaged is that phytomining can be farmed out to small-scale landholders and farmers throughout the region where low-grade metalliferous soils are present. Small-scale operation can be environmentally sustainable because usually small farms are farmed directly by the landholder, which is therefore interested to maintain or increase the fertility of his/her land. Higher revenues can be obtained if farmers unite in co-op to reduce the costs of mechanisation and transportation by economy of scale.

Fertility of land is likely to be increased after phytomining operation because of increased root and microbial activity in the soil profile, Ni removal, and pH and fertility management. Therefore, landholders may want to use land for food production after phytomining has reached Ni depletion of the top layers and therefore reduced Ni toxicity to crops occurs. The possibility of restoring serpentine soils post-phytomining and convert those soils to food production is an important aspect of environmental sustainability of phytomining and needs to be addressed in future studies.

5 Conclusions

The study and development of phytotechnologies in the past 30 years have produced important advances in understanding fundamental aspects of physiology, ecology, and agronomy of hyperaccumulator plants and biogeochemistry and ecotoxicology of TEs. However, the application of the phytoextraction technology to real-world situations has been deterred by the lack of understanding that the complexity of biological systems, which are put to 'work' to provide a service to humans, can hardly fit the constraints of market economy (i.e. time efficiency and high revenues). As discussed above, the main constraints to the commercialisation of phytotechnologies and in particular phytoextraction are the time needed to clean up the soil and the costs associated with production and disposal of biomass.

A more interdisciplinary approach is needed to understand the multi-facets of introducing a designed biological system, made of specialised plants and trees, into an area of land that has been environmentally damaged. Studies on the ecological assessment, environmental sustainability, socio-economic aspects of rural development and improvement of local economies, and life cycle assessment are very important and need to be performed in any revegetation and phytoextraction plan. A complete analysis of the direct and indirect costs and benefits of the application of phytotechnologies is very important to gain a deeper understanding of the complexity of such a system. Consequently, the cost/benefit analyses of phytotechnologies will not only rely on principles of market economy (i.e. time efficiency and high revenues) but also on indirect environmental and socio-economic benefits to society. When future studies are able to provide a description of the multi-facets of phytotechnologies, a stronger support will be gained from local communities and the public at large, which will then trigger government support and financial aid.

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Integrated Management of Mine Waste Using Biogeotechnologies Focusing Thai Mines

M.N.V. Prasad and Woranan Nakbanpote

Abstract

The history of mining for precious minerals dates back to several centuries. Mining is important for economy but causes environmental contamination. However, mine waste reclamation and mine environment cleanup are a subject of recent origin focusing various aspects of biogeotechnologies. In general, the subject of environmental remediation is about three decades old, and today the advances in this field are capable of handling a variety of toxic waste. Different strategies and approaches are employed to render mine waste less toxic. Mining had negative effects on natural resources (biotic and abiotic) and deteriorates the quality of environment. Different types of mine industries are implicated in promoting "industrial deserts" or "lunar scapes" which are overloaded with technogenic waste. Soil washing and cleaning in such situation is cost prohibitive. This chapter deals with reclamation of a zinc, lead, and tin (arsenic) mine waste with reference to Thailand (see graphic abstract Fig. 1).

1 Introduction

Extraction of metals by the mining industry generates large amounts of waste material, which has to be taken care of to prevent negative effects on the environment. Once metals are extracted from the ore, the rest of the finely grained soil is pumped out as wet slurry (tailing ponds). The problem with mine tailings is that it still contains high levels of heavy metals that could be spread

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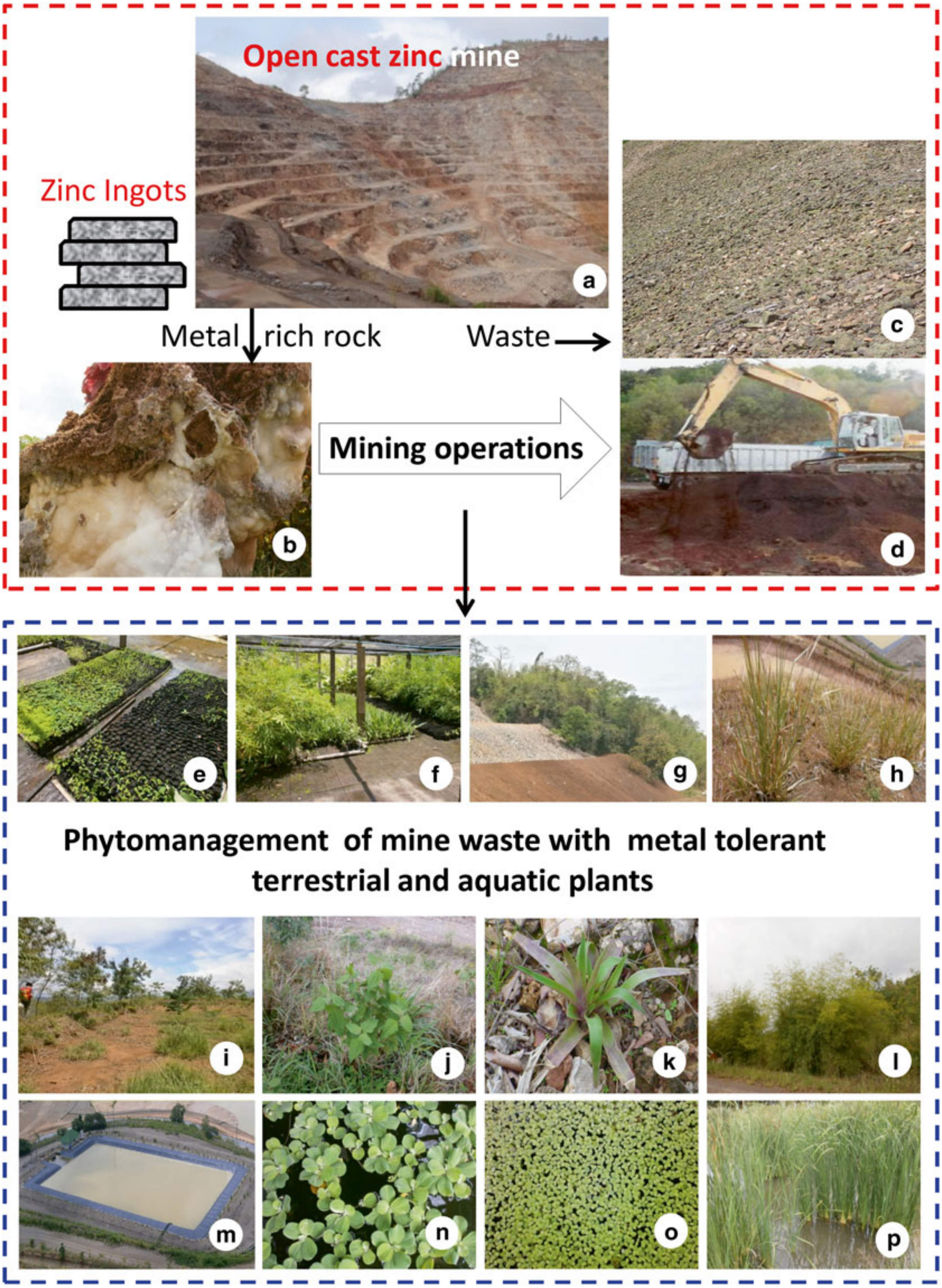

Fig. 1 Graphic abstract depicting the integrated management of mine waste using biogeotechnologies

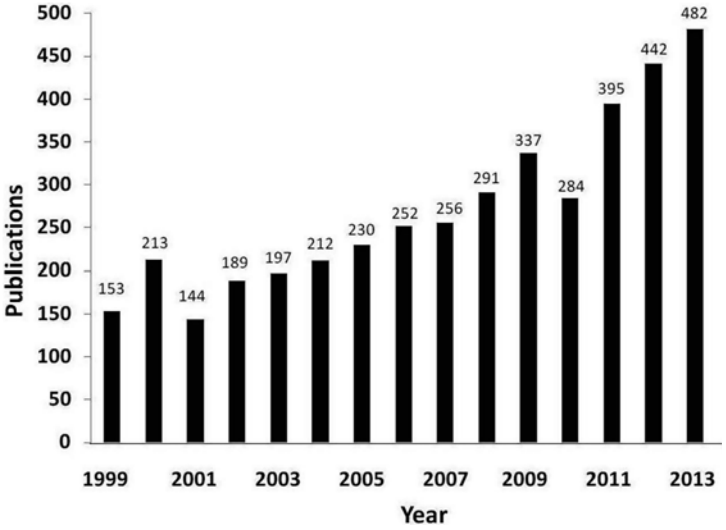

Fig. 2 Knowledge explosion in mine waste reclamation (Data gleaned from www.sciencedirect.com with keyword "mine reclamation")

and transported to different places with the wind or leached with water. If the metal-rich parent rocks consist of iron- and sulfur-containing mineral pyrite, this sand is often rich in sulfur that by reaction with oxygen and water is transformed to sulfuric acid. Thus, the tailings are subjected to chemical weathering process leading to the formation of acidic mine drainage in which heavy metals easily dissolve. To avoid leakage of heavy metals, the mine residue needs to be covered with a material that prevents penetration of oxygen. There has been knowledge explosion in mine waste reclamation (Fig. 2) (Li 2006). A relatively cheap method is to cover the tailings with water; however, this method demands barriers that must be carefully maintained to avoid the risk of broken walls and thereby contamination of the surroundings. Therefore, many mine tailing impoundments are instead covered with a dry material (back filling from mine source). In restoration of mine areas, the establishment of vegetation is very important, not only for giving the landscape a natural appearance but also for stabilization of the cover material (Wong 2003; Zornoza et al. 2012).

Environmental implications of mine waste:

(a) Threat to biodiversity
(b) Generate acid drainage
(c) Food safety in the vicinity of mine
(d) Threat to national heritage
(e) Increase environmental pollution in soil, water, and air

2 Ecological Restoration of Mine Wastes

Various unit operations of "ecological restoration of mine waste" are shown in Fig. 3. A layer of vegetation can prevent both physical erosion and leaching of heavy metals.

2.1 Covering Techniques

Weathering of mine tailings can be prevented by a minimized contact with air, which can be done by covering the tailings with either water (wet cover) or a dry substrate (dry cover).

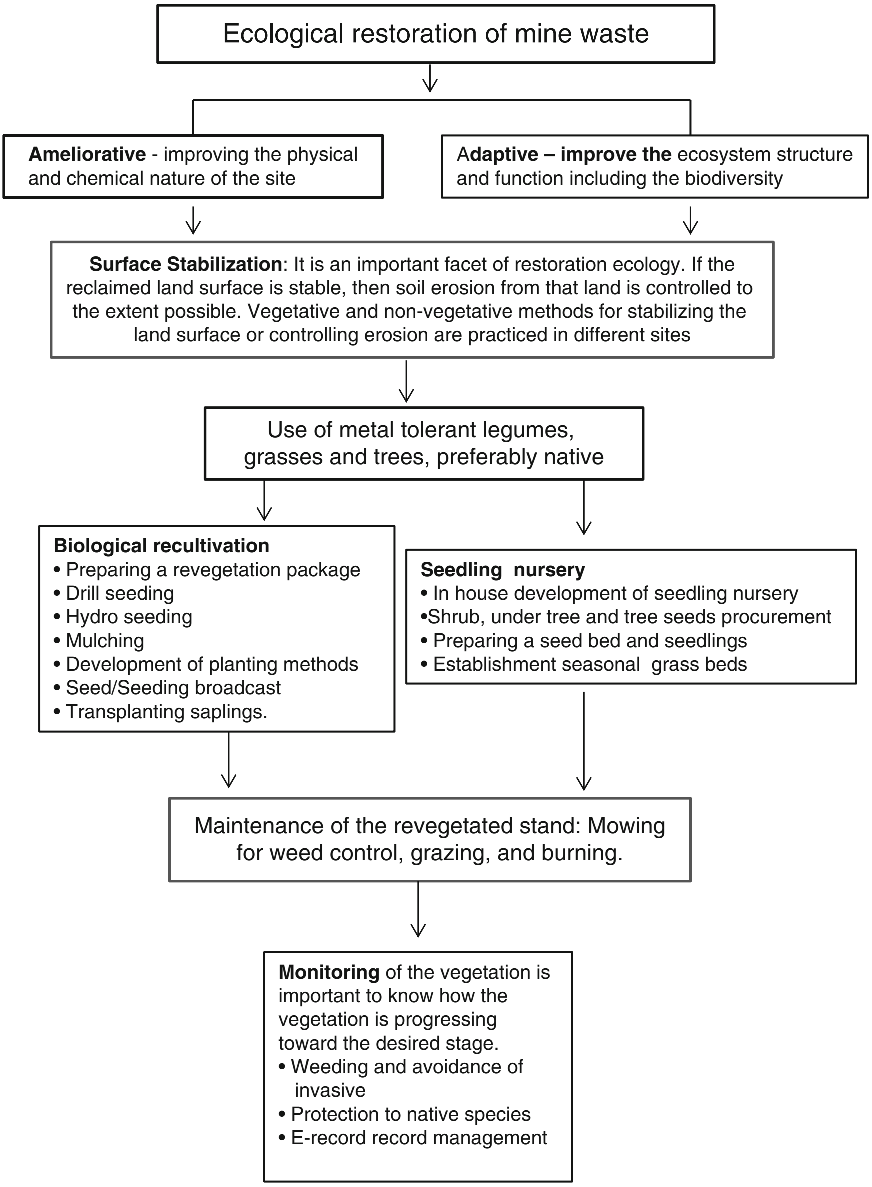

Fig. 3 Schematic presentation showing various unit operations of "ecological restoration of mine waste" (Badiozamani and Askari-Nasab 2014; Kalin and Wheeler 2011; Stokes et al. 2010)

2.1.1 Dry Covers

Dry covers can prevent weathering by acting as a barrier against oxygen. Apart from that, a dry cover can also facilitate the recovery of the area toward a more natural ecosystem and a locally adapted solution of vegetation establishment (Bradshaw 2000; Chen et al. 2013). Dry cover can be based on only one material or a combination of different substrates coupled with engineered vegetative capping technology (Fig. 4a, b) (Prasad 2014).

Traditionally the most common method is to use a thin layer of cover consisting of moraine, but since the nutritious values are low and the extraction of moraine is expensive, new materials are under evaluation. To function as an efficient

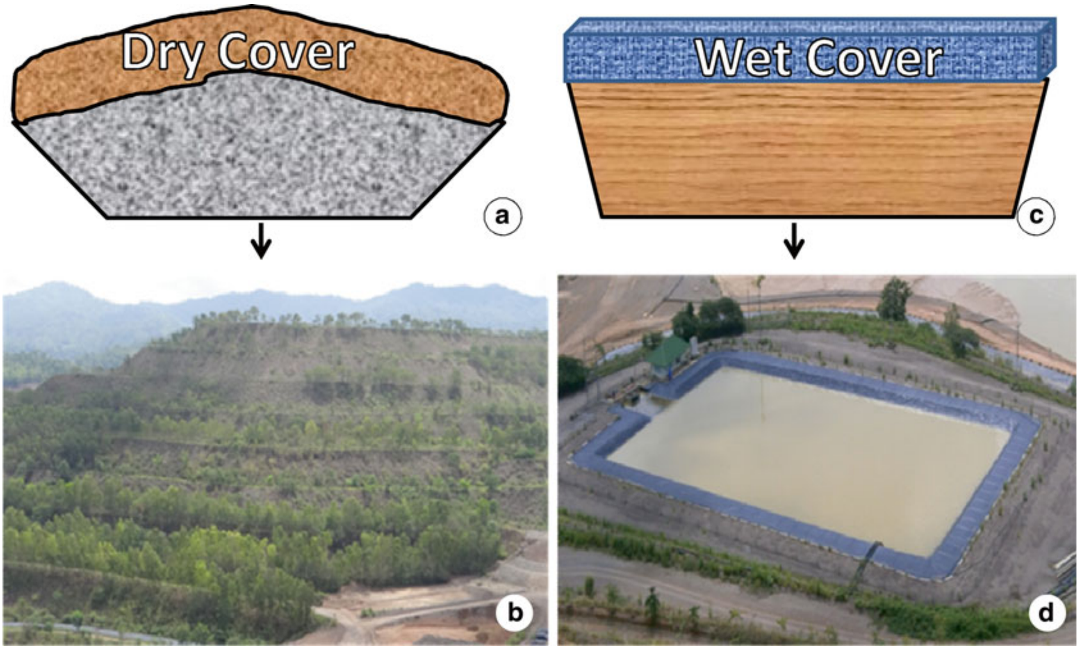

Fig. 4 Mine tailings (**a**) schematic dry cover, (**b**) zinc mine waste dry cover, (**c**) schematic wet cover, (**d**) zinc mine waste in covered with water, Padaeng zinc mine

barrier against oxygen, the material must have a high capability to keep water, with the ability to consume oxygen consisting of an organic material, such as sewage sludge or paper mill sludge.

2.1.2 Wet Covers

The tailings are flushed into water body with a water column over and above the mine waste. The depth of the water column must be adequate to avoid oxidation. Preferably the wet cover treatment is established on fresh tailings that are not yet oxidized. Flooding of pre-oxidized tailings must be avoided in order to prevent the release of metals (Fig. 4c, d) (Demers et al. 2008; Holmström and Öhlander 2001; Macías et al. 2012).

2.2 Sodding

It is a bioengineering technique that uses vegetation mats for mine soil stabilization and erosion control. This technique has been used with success with a variety of grasses (Fig. 5). Perennial grasses with sturdy adventitious root mat anchor and stabilize mine soils. The following is a list of recommendations for using vegetation mats as bioengineering materials:

- To anchor perennial solid binding grass mats to a slope, mats can be cut to form any shape desired. A shallow, narrow trench built along the contour of a slope and planted with a vegetation mat may become an effective terrace.
- The mat should remain attached to stable vegetation and thus be held in place from the top. The mat can be pegged to prevent ripping and sliding. This technique would be used to stabilize the contaminated soil.
- Vegetation mats can be used as building bricks. Slice the mats into rectangular pieces and use them to construct a very steep, living wall. The bricks can be pegged to each other and to the underlying substrate. This technique may be useful around culverts or sunken walkways and control erosion.

In Australia, *Vetiveria zizanioides* has been successfully used to stabilize mining overburden and highly saline, sodic, magnesic, and alkaline (pH 9.5) tailings of coal mines, as well as highly acidic (pH 2.7) and arsenic (As) tailings of gold mines. In China, it has been demonstrated that

Fig. 5 Sodding, a bioengineering technique that uses grass mats for soil stabilization and erosion control. Perennial grasses are preferred. Grasses with sturdy adventitious root mat stabilize metalliferous soil. To anchor perennial solid binding grass mats to a slope, mats are cut to desirable sizes (1 × 1 ft). This technique would be beneficial to stabilize the contaminated soil

V. zizanioides is one of the best choices for revegetation of Pb/Zn mine tailings due to its high metal tolerance (Chen et al. 2004; Truong 2004).

3 Phytomitigation of Abandoned Mines and Mine Waste

Thailand is rich in metal resources such as gold, zinc, silver, iron, tungsten, tin, antimony, copper, and lead (Table 1). Thailand is challenged with three important environmental problems due to mining activity as (1) arsenic (As) contamination in Ron Phibun District, Nakhon Si Thammarat Province; (2) lead (Pb) contamination in Klity Creek in the Thung Yai Naresuan Wildlife Sanctuary, Thong Pha Phum District, Kanchanaburi Province; and (3) highly elevated cadmium (Cd) concentrations in paddy soil in the Mae Sot District, Tak Province (Fig. 6). Therefore, the Department of Primary Industries and Mines (DPIM), a governmental organization responsible for mining and mineral processing, prepared technical guidelines for environmental management and rehabilitation. These guidelines concentrate on using biogeotechnologies for reclamation of mine waste, green mining policy, and sustainable development (Table 2).

Table 1 Metal resource in Thailand

Metal	Metal reserved under patent permit (dmt)	Metal reserved in potential resource (dmt)	Metal remaining in potential resource (%)
Gold	26	172	84.88
Zinc	3,796,190	5,796,190	34.51
Silver	96	526	81.75
Iron	41,068,954	191,068,954	78.51
Antimony	1,246	50,001,246	99.99
Copper	0	1,000,000	100.00
Lead	0	800,000	100.00

Source: Department of Primary Industries and Mines (DPIM) in 2008
Data at the end of 2005
dmt dry metric tons

Integrated biogeotechnologies for reclamation of mine waste are shown in Fig. 7 (Benzaazoua et al. 2008). In recent times, application of phytotechnologies for restoration of contaminated sites is gaining considerable significance. Establishment of vegetation is not only aiming to return the area to its original appearance but also to prevent the leakage of toxic metals (Bradshaw 1997). Dealing with mine waste requires integrated package of practices. A careful selection of plants that do not transport the metals to the harvestable parts like shoots is desirable as these would lockup the metals in the root or the rhizosphere (= phytostabilization). Apart from the physical stabilization that protects against erosion, the roots have a beneficial effect on mine tailings through decreasing acidity and oxygen concentration, and they prevent rainwater from percolating to the tailings.

Numerous approaches are being followed to establish viable plant covers directly on mine tailings (Bell 2001). However, since the impoundments offer a very harsh environment to the plants, with high levels of heavy metals, low levels of macronutrients, and poor substrate structure, these attempts usually have failed, and the process is rather very slow in establishment of plant cover. Natural succession is possible even on such areas, but a time span of at least 50–100 years is needed before a viable vegetation cover is developed (Bradshaw 1997).

Establishment of plants can take place through natural spreading if the restored site is small and surrounded by natural vegetation, a low-cost method that would enhance natural appearance (Prach and Pyšek 2001). However, when the sites are large and heavily polluted, like mine waste deposits, natural succession will take a considerably longer time (Bradshaw 2000). To get a faster and more homogenous establishment of vegetation, planting and sowing are often practiced, which should be performed repeatedly to give satisfactory vegetation (Bradshaw 2000). Important for a sustainable reclamation of a soil ecosystem and healthy vegetation is also to establish a functioning microflora. If the covering material is low in nitrogen, colonization of nitrogen-fixating bacteria is valuable, but as the nitrification process generates acidity, favoring of these bacteria might be disadvantageous for the reclamation of already acid mine tailings. The use of paper mill sludge, an organic material with low nitrogen levels, has shown to be a well-functioning cover material if used in combination with initial fertilizers and establishment of leguminous plants.

4 Mitigation Strategies for Arsenic Contamination in Ron Phibun

Thailand's well-known hot spots for As contamination are located in the northwest part of the town in Ron Phibun District, Nakhon Si Thammarat Province, in the southern part of Thailand. It lies between 8° 04′ and 8° 14′ N latitudes and between 99° 46′ and 99° 54′ E longitudes (JICA 2000). The second site is Nakhon Si Thammarat Province in the Ongpra Subdistrict,

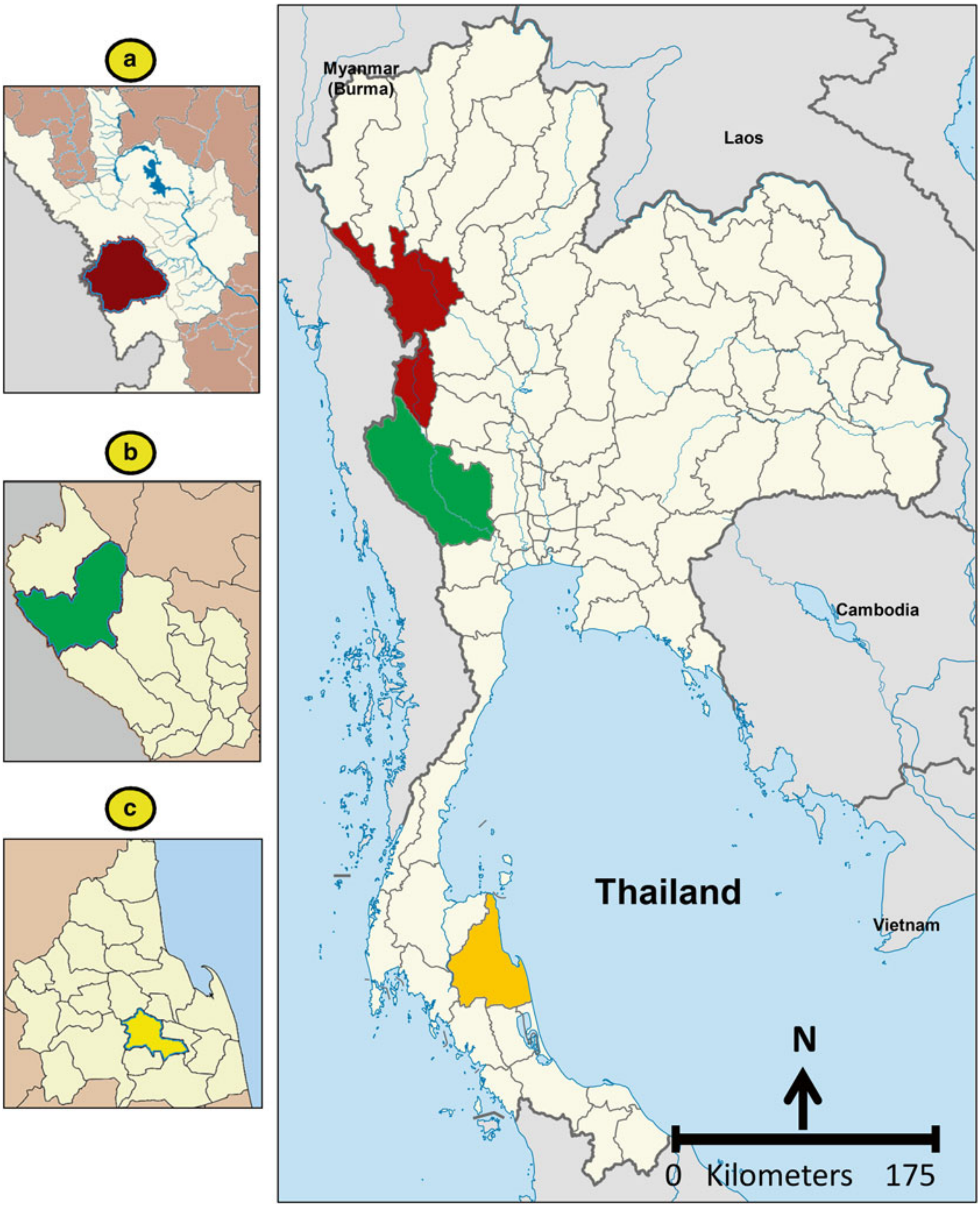

Fig. 6 Three important environmental problems due to mining activity in Thailand. (**a**) **Zn** mining in Mae Sot District, Tak Province, (**b**) **Pb** mining in Thong Pha Phum District, Kanchanaburi Province, and (**c**) **Sn** mining in Ron Phibun District, Nakhon Si Thammarat Province

Dan Chang District, Suphanburi Province, 14° 50′ N latitude and 99° 42 E longitudes. Mine reclamation is done (Fig. 8) with selected plants (Tanpibal 1989).

Tin (Sn) mineralization from cassiterite in (SnO_2) Ron Phibun District, Nakhon Si Thammarat Province, contains approximately 1 % arsenopyrite (FeAsS) and pyrite (FeS_2).

Table 2 Arsenic accumulation in ferns

Name of the fern	As (mg kg^{-1})	References
Pteris vittata	22,630	Ma et al. (2001)
Pityrogramma calomelanos	8,350	Francesconi et al. (2002)
P. umbrosa	7,600	Zhao et al. (2002)
P. cretica	3,030	Zhao et al. (2002)

The site is part of the Southeast Asian tin belt, an area that has a 100-year history of bedrock and alluvial mining. Soil and groundwater in the area were contaminated with up to 0.1 % arsenopyrite and 100 times of the regulatory arsenic level of 0.01 mg/L in drinking water (Mandal and Suzuki 2002). People live near or in old tin mine area were sick, and illness includes chronic As poisoning (arsenicosis), skin cancer, and bladder cancer, resulting from drinking As-contaminated water (Choprapwon and Porapakkham 2001). The high concentrations of toxic inorganic arsenic (predominantly arsenate) in an edible fish *Channa striata* had human health implications and warrant wider investigations (Jankong et al. 2007). The As was weathered from arsenopyrite by both natural and mining activity, which leaches out and contaminates local soil and groundwater (William et al. 1996). Although tin mining was prohibited on February 4, 1994, the release of arsenopyrite from the tin ore has left extensive damage. The point source of waste piles (2,500 m^3, approximately) had to be landfilled secure at Suangchan-Ronna mountain, Khao Luang Naional Park, in Ron Phibun District, and the old tin mine had to be reclaimed and remediated. Monitoring program detects As contamination in river, water supply, and soil at least once in 2 years. Constructed wetland and phytoremediation to remediate the mine tailing and the As-contaminated site are a promising option. Two species of ferns (*Pityrogramma calomelanos* and *Pteris vittata*), an herb (*Mimosa pudica*), and a shrub (*Melastoma malabrathricum*) and about 36 plant species are recommended as potential candidates for phytoremediation (Visoottiviseth et al. 2002). The root uptake of K, P, and S was significantly reduced when ferns were exposed to As, but the translocation factor increased in most ferns to maintain nutrient requirements and ion balance (Sridokchan et al. 2005). Phosphate fertilizer significantly increased plant biomass and As accumulation in *P. calomelanos* (silverback fern) when grown in As-contaminated soil. P-fertilizer and rhizosphere bacteria enhanced As-phytoextraction, whereas rhizosphere fungi exerted their effects on phytostabilization (Jankong et al. 2007). The toxicity of different forms of As decreases in the following order: arsine> inorganic arsenite> organic arsenite> inorganic arsenate> organic arsenate> free arsenic (Mandal and Suzuki 2002). Some algae, fungi, and bacteria have the ability to transform arsenite to arsenate. The mechanisms involved in the microbial transformation and removal of As from the environment included adsorption and reduction reaction (Anderson and Cook 2004). Arbuscular mycorrhizal fungi (AMF) can play an important role in phytoremediation. The principle role of mycorrhizal fungi is obtaining phosphorus (P) for their hosts. Hence, enhanced acquisition of phosphate may also lead to enhanced acquisition of arsenate (Meharg and Hartley-Whitakar 2002). However, the effect depends on the interactions between AMF and their host plants. Inoculation of AMF (*Glomus mosseae*, *Glomus intraradices*, and *Glomus etunicatum*) reduced As accumulation in *P. calomelanos* and *Tagetes erecta* and had no effect on the plants growth. In contrast, the AMF improved growth and As accumulation in *M. malabathricum* (Jankong and Visoottiviseth 2008). Effects of AMF on As accumulation by plants were also influenced by root morphology. *P. calomelanos* and *T. erecta* possess wide-root system with a fine network of roots that enhance phytostabilization. AMF also enhanced the growth of *M. malabathricum* because of the plant's root structure (Jankong and Visoottiviseth 2008).

The fern has the capability to accumulate 5,000 µg As/g dry mass of fronds, assuming the soil contains 500 µg As/g with the density of 2 g/cm^3. The fern's root system is able to reach 25 cm depth; a plant this size will have approximately 50 g dry mass of fronds; and 16 plants per m^2 can be cultivated/harvested each year. The annual

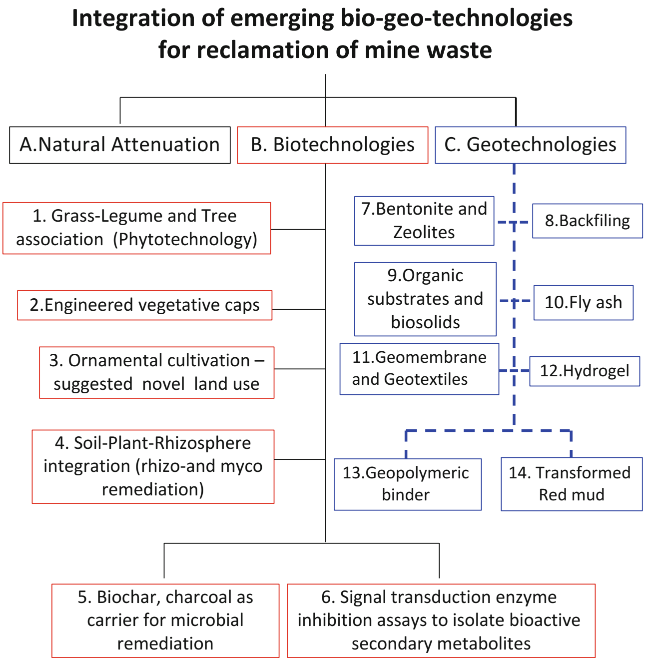

Fig. 7 Integration of emerging biogeotechnologies for reclamation of mine waste (*A*) Natural attenuation (Arienzo et al. 2004; Asensio et al. 2013a, b; Bell 2001; Bidar et al. 2007; Bech et al. 2012; Bradshaw 1997; Chiu et al. 2006; Drahota et al. 2012; Valente et al. 2012) (*B*) Biotechnologies *1* – Grass-based phytomanagement (Pang et al. 2003; Tamang et al. 2008), *2* – Engineered vegetative caps (Kim and Benson 2004), *3* – Ornamental cultivation – suggested novel land use (Chintakovid et al. 2008), *4* – Soil-plant-rhizosphere continuum (Fitz and Wenzel 2002; Solís-Domínguez et al. 2011), *5* – Biochar (Bian et al. 2013; Ahmad et al. 2014), *6* – Use of signal transduction enzyme inhibition assays to isolate bioactive secondary metabolites acid mine waste lake (Stierle and Stierle 2013), (*C*) Geotechnologies *7* – Zeolite (Fall et al. 2009), *8* – Backfilling (Benzaazoua et al. 2008), *9* – Organic substrates (Dobran and Zagury 2005; Kijjanapanich et al. 2014; Macías et al. 2012; Santibáñez et al. 2007, 2008; Zornoza 2012; *10* – Fly ash (Ram and Masto 2010; Zhang et al. 2011), *11* – Geomembranes and geotextiles (Lupo and Morrison 2007), *12* – Hydrogel (Bigot et al. 2013), *13* – Geopolymeric binder (Pacheco-Torgal et al. 2008), *14* – Transformed red mud (Ardau et al. 2013; Cappai et al. 2012) and Red Clay (Mopoung and Thavornyutikarn 2006)

harvested biomass (fronds only) will be 800 g/m^2, which will contain a total of approximately 4 g of As. The reduction in soil As concentrations might be in the order of 2 % per year in the example quoted (Francesconi et al. 2002). The As-rich plant will itself need to be dealt with. The type of inorganic arsenic compounds [arsenate, As(V) and arsenite, As(III)] is present in the fern. The As(III) was a predominant form in excised aerial tissues, whereas the As(V) was the main form in excised roots clearly demonstrated that As reduction occurred mostly in the fronds mainly in the pinnae (Tu et al. 2004). Combustion of such a waste product is not a feasible option because it would likely lead to release of toxic As_2O_3. Disposal of the high As in fern biomass directly into the open sea where it would degrade (contribution marginally to nutrient levels) and release inorganic As which could be converted to a nontoxic form of arsenobetaine by natural processes might be a feasible option (Francesconi et al. 2002).

Considering the needs of the local people, an important criterion for selection of the potential plants for phytoremediation could be provision of economic benefits to the remediations, and nonedible. *P. calomelanos* has the ability to accumulate As; however, this fern is edible in Thailand (Francesconi et al. 2002). Therefore, the use of ornamentals for, e.g., marigold, a triploid

Fig. 8 Mine reclamation with selected native Thai plants (Prasad et al. 2014)

hybrid between American (*Tagetes erecta* L.) and French (*Tagetes patula*) marigolds, are potential candidates for field remediation of soils contaminated with As in Ron Phibun District, Nakhon Si Thammarat Province, southern Thailand (Chintakovid 2008, Francesconi et al. 2002; Huq et al. 2005). Marigolds have a high potential for As phytoremediation being nonedible and income generators. The waste generated from such ornamentals after decay is relatively easier to dispose off in secure landfills (Prasad 2012). These studies have provided critical information for better understanding of phytoremediation of As-contaminated soils in Thailand.

4.1 Phosphate Fertilization of Arsenic-Contaminated Soils

Arsenic is toxic whereas phosphorus (P) is essential for plants. Phosphate is reported to suppress plant uptake of arsenate. Studies have also shown that at low levels, arsenate can increase phosphate uptake (Cao et al. 2003). They are both having similar electron configurations and chemical properties. Therefore, arsenate and phosphate will compete with each other for soil sorption sites, resulting in a reduction in their sorption by soil and an increase in solution concentrations. Phosphate significantly suppressed the sorption of arsenate. Phosphate fertilization of As-contaminated soils seems to be one of the feasible strategies used for successful phytoremediation. Interactive effects between arsenate and phosphate have been reported by Gao and Mucci (2003). Arsenate may replace phosphate in ATP synthesis and/or in various phosphorolysis reactions, thus interfering with phosphate metabolisms and causing toxicity to a plant (Dixon 1997). In contrast, phosphate may be able to alleviate arsenate toxicity by improving phosphate nutrition (Sneller et al. 1999). *Pityrogramma calomelanos*, an As-hyperaccumulating fern, has been suggested as a potential phytoremediator of As-contaminated soils in Thailand.

4.2 Rhizospheric Exudates Induce Tolerance to Arsenic Toxicity in Plants

Root exudates (metabolites that are released to the root surface) are generally classified into two types, viz., high molecular weight (HMW = polysaccharides and polyuronic acid) and low molecular weight (LMW = organic acids, sugars, phenols, and various amino acids, including nonprotein amino acids such as phytosiderophores) (Marshner 1995). The composition and quantity of root exudates vary from plant to plant with two factors being important. One is a plant's inherent biology, such as plant species, growth and developmental period, and nutrient status. The other is the external environment for plant growth, i.e., soil and its elemental content (Gregory and Atwell 1991).

Numerous researchers have probed into the identification, characterization, and functions of root exudates and their movement in the rhizosphere (Li et al. 2013). Since soil metals/metalloids are often insoluble and unavailable for plant uptake, plants that possess such mechanisms to reduce the bioavailability of metals and metalloids in the rhizosphere have the potential for reclamation. Arsenic waste dump sites in Thailand were colonized by *M. pudica* and several varieties of bent grasses including colonial bent grass (*Agrostis tenuis*).

4.3 Grasses as Ideal Plants for Remediation of Arsenic-Contaminated Soils

Certain Poaceae members (grasses) such as *Agrostis castellana*, *A. delicatula*, and *Holcus lanatus* have played significant role in revegetation of the As-contaminated soil in Southwest Europe (De Koe et al. 1994). Arsenic accumulation was found in all parts of the grasses with different levels of concentration. *Vetiveria zizanioides* is a perennial grass with strong ecological adaptability and large biomass and is easy to manage and grow in different soil conditions. It has great potential for various reclamation applications. Vetiver is an extremely hardy grass species with many characteristics that makes it ideal for environmental protection, i.e., its roots reach 3–4 m in ideal conditions. The application of vetiver grass was first developed by the World Bank for soil and water conservation in India and Thailand. In Thailand, two types of vetiver grass such as *V. zizanioides*, Surat Thani ecotype, and *V. nemoralis*, Prachuap Khiri Khan ecotypes, are easily found. They have the ability to grow well in various climates and in the different geographical areas present in Thailand. *V. zizanioides* can tolerate and grow in high metal-contaminated soil (Truong 2004). The As accumulation in both ecotypes of vetiver grass was higher in the roots than the leaves (Tlustoš et al. 1997). In greenhouse experiments, Heeraman et al. (2001) observed that the interactions of lime, N, P, and organic matter (OM) additions to As-contaminated soil with respect to plant growth promoted Zorro fescue (*Vulpia myuros* L.) growth.

4.4 Prospects of Arbuscular Mycorrhizal Fungi in Phytoremediation of Arsenic-Contaminated Soils

Arbuscular mycorrhizal (AM) fungi are reported to be the integral constituents of plant root systems in toxic trace element-contaminated soils (Gaur and Adholeya 2004; Luo et al. 2014). A result of a

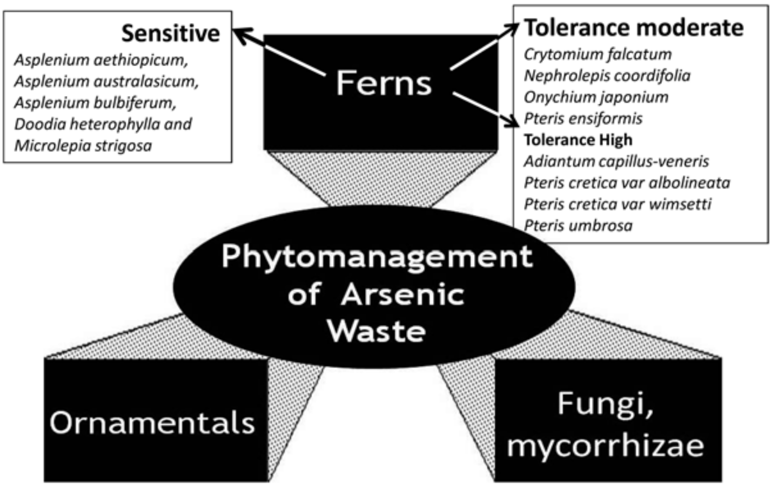

Fig. 9 Phytomanagement strategies of arsenic waste management with ferns, ornamentals, fungi, bacteria, and mycorrhizae

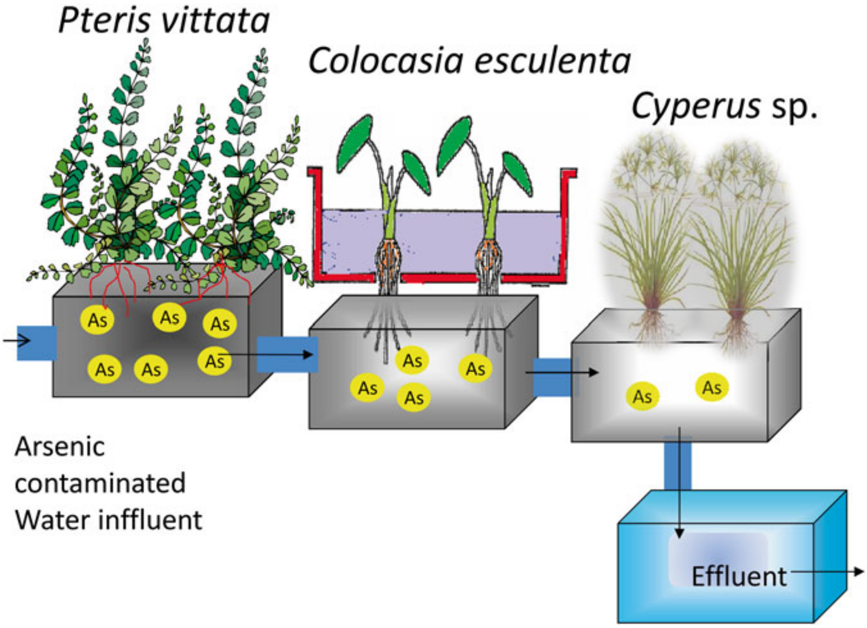

Fig. 10 Use of arsenic accumulator plants (*Pteris vittata*, *Colocasia esculenta*, and *Cyperus* sp.) for arsenic removal from contaminated water (Kurosawa et al. 2008; Nakwanit et al. 2011)

colonization by an AM fungus (*Glomus mosseae*) on biomass and an arsenate uptake of an As hyperaccumulator, *Pteris vittata*, has been reported (Wu et al. 2009). Arsenic-hyperaccumulating fern, *P. vittata* (an important foliage ornamental), was also used in the pilot-scale demonstration phytofiltration project to produce drinking quality water from As-contaminated groundwater in New Mexico, a classic example of service to mankind (Elless et al. 2005) (Figs. 9 and 10).

Plants forming mycorrhizas can grow on As-contaminated soils (Meharg and Hartley-Whitaker 2002; Sharples et al. 1999, 2000a, b). High concentrations of As inhibited the growth of the ericoid mycorrhizal fungus *Hymenoscyphus ericae* (Sharples et al. 2000a). Inoculation with the AM fungus led to a decrease in the As concentration in the host plants and an increase in dry matter yield, especially of the fronds. Although the As concentrations in the fronds were lower at the high As application rate, inoculated treatments had higher biomass, giving larger amounts of As uptake by the fronds of mycorrhizal plants. The enhanced biomass with mycorrhizal inoculation was associated with enhanced P uptake and consequently increased the quantity of As removed from the soil by the hyperaccumulator (Koltai and Kapulnik 2010). Further, AM fungi acquire P for their host plants, but this may lead to problems on arsenate-contaminated substrates if enhanced acquisition of phosphate is accompanied by enhanced acquisition of arsenate. Since arsenate can enter plants through their phosphate transporters (Lee 1982), mycorrhizal fungi may enhance uptake of both phosphate and arsenate by plants.

Arsenic resistance of mycorrhizal plants might be partly explained by the higher P/As ratios in mycorrhizal plants. Thus, mycorrhizal plants obtained more P than non-mycorrhizal controls, and the higher P/As ratios may be one of the mechanisms by which AM fungi can enhance host plant resistance to As, even in the case of As hyperaccumulators. Many studies have indicated positive effects of mycorrhizas on host plants under environmental stresses could be directly or indirectly attributed to improved plant P nutrition (Koltai and Kapulnik 2010). However, there were significant increases in root P concentration with mycorrhizal colonization. In general, the phosphate analogue arsenate is more toxic to plants than arsenite. On the other hand, arsenate reductase, which catalyzes the reduction of arsenate to arsenite, is expressed in leaves only and not in roots (Dhankher et al. 2002). Since more P was stored in the roots with increasing As application and mycorrhizal colonization enhanced this trend, we suggest that the higher P concentration in the roots may contribute to As resistance in the mycorrhizal plants. Phytotoxicity of arsenate has been observed under conditions of low P supply (Wang et al. 2010), and mycorrhizal inoculation has been observed to alleviate As phytotoxicity by increasing plant P content.

Although tin mining was stopped in the late 1980s, the release of arsenopyrite (FeAsS) from the tin ore has left extensive As contamination in many areas of Ron Phibun District. The long-term use of contaminated water for agriculture or direct consumption poses a serious risk of chronic As poisoning among the local population causing diseases such as skin melanoma, cancer, and high blood pressure. The environmental problems originating from As contamination is still observed. Therefore, to protect animal and human health, remediation of the As-contaminated sites has been a priority area of research and development. The Ron Phibun wasted soil is a highly complex, heterogeneous mixture of sulfide, silicates, and oxide with high total concentrations of As (Arrykul et al. 1996). Use of *P. calomelanos* for phytoextraction of As appears to be a feasible solution (Alkorta et al. 2004).

5 Lead Contamination in Klity Creek and Reclamation

Lead (Pb) in Thong Pha Phum District, Kanchanaburi Province are primary deposit of lead sulfide (galena, PbS) in stratabound deposit and secondary deposit (cerussite, $PbCO_3$). The secondary deposit of lead, which is changed from lead sulfide due to oxygen and underground water, is in topsoil and subsoil. In 1998, torrential rain caused the mine's tailings facility to rupture, releasing more than 800 metric tons of Pb into the Klity Creek watershed, Thong Pha Phum District, Thailand. The mine and processing facility were immediately shut down, and the Public Health Ministry prohibited using water from the creek and banned fishing (Nobuntou et al. 2010; The Nation 2013). The Pb tailing was excavated from Klity Creek in 2.5 km distance during 1999–2000. The lead sludge was landfilled in tailing pond. The eight ponds, which were not in the standard of secure landfill, were far from the creek 5–10 m, with slope 10–20°. To protect the leachage of Pb into the creek and decrease the contact of lead sulfide ores with oxygen, the ways that the Pollution Control Department (PCD) suggested are to maintain the tailing pond by covering with geomembrane HDPE and 30 cm of top soil before growing covering plants such as *Wedelia trilobata* (L.) Hitchc. The tailing ponds were surrounded with pass channel and vetiver grasses. Some rock check dam was constructed in the creek to precipitate small particle of Pb tailing (Water Quality Management Bureau, PCD, 2009). However, environmental monitoring illustrated that the Pb concentration in the surface sediment was as high as 869.4 mg/kg, whereas in agricultural soil ranged between 137 and 613 mg/kg of Pb and was inversely proportion to the distance from the point source. Moreover, Pb was transported from the point source to downhill areas. Vegetables (mint, bitter gourd, Chinese watercress, basil, and turmeric), tuber crops (cassava and curcumin), and meat (fish and shellfish) had Pb concentrations above the recommended standard (Pusapukdepob et al. 2007). Karen villagers, an indigenous community, were leading a simple life interconnected with the surrounding nature until the mine released Pb-contaminated wastewater into the creek. The villagers suffered due to Pb poisoning and contamination in the creek (Assavarak 2012). Health risk assessment of the villagers have been reported in low intelligence quotient (IQ) score, and their blood Pb or tooth Pb levels were higher than normal limit (Pusapukdepob et al. 2007). In January 2013, a Thai court ordered the government to clean up the creek and compensate the villagers (The Nation 2013). Klity Creek is in the Thung Yai Naresuan Wildlife Sanctuary; therefore, this Pb-contaminated area needs to be remediated.

A total of 48 plant species belonging to 14 families were collected from a closed open-pit Bo Ngam lead mine area, Thong Pha Phum District, which Pb concentrations in surface soil ranged from 325 to 142,400 mg/kg. Three species (*Microstegium ciliatum*, *Polygala umbonata*, and *Spermacoce mauritiana*) showed extremely high Pb concentrations in their shoots (12,200–28,370 mg/kg) and roots (14,580–128,830 mg/kg) (Rotkittikhun et al. 2006). Most plants had the highest Pb content during the wet season (May to September) and the lowest during the dry season (October to April). There were a total of 17 plant species that had Pb accumulation in shoots >1,000 g/kg, though only six species (*Ageratum conyzoides*, *Buddleja asiatica*, *Chromolaena odorata*, *Conyza sumatrensis*, *Mimosa pudica*, and *Sonchus arvensis*) showed a translocation factor >1 (Homyog et al. 2008). The Siam weed, *Chromolaena odorata*, belongs to Asteraceae is a hyperaccumulator by means of field surveys on Pb soil around Bo Ngam Pb mine and hydroponic studies, which grows rapidly and has substantial biomass. This perennial herb with great ecological amplitude possesses great potential for use in the remediation of Pb-contaminated soil (Tanhan et al. 2007). Greenhouse and field trial experiments were performed to evaluate the use of *C. odorata* with various soil amendments for phytoextraction of Pb-contaminated mine soils, which contain low amount of nutrients. Inorganic fertilizer (Osmocote) enhanced Pb accumulation; cow manure decreased available Pb concentration and resulted in the highest Pb concentration in roots; ethylenediaminetetraacetic acid (EDTA) increased Pb accumulation in shoots and roots (Tanhan et al. 2011). *S. arvensis*, a pioneer perennial plant, is a good candidate for Pb phytoremediation. In pot study, *S. arvensis* grown in Pb mine soils amended with organic fertilizer and EDTA has the highest Pb shoot accumulation (1,397 mg/kg), and the plants accumulated Pb in the shoot up to 3,664 mg/kg when grown in a field experiment in the mine area (Surat et al. 2008). The use of EDTA in the field should be concerned with their leaching problems (Tanhan et al. 2011).

A field survey at Bo Ngam Pb mine found 11 fern species including *P. vittata* which accumulated Pb in the range of 23–295 mg/kg in the aboveground parts. When *P. vittata* was grown at mine soils for 6 months, the fern tolerated higher soil Pb (94,584–101,405 mg/kg) and accumulated

Pb in frond (4,829 mg/kg) after 2 months of growth (Soongsombat et al. 2009). The high Pb accumulation and tolerance indicated that *P. vittata* can be potentially useful for phytoremediation and phytomining of Pb-contaminated soil.

A glasshouse study was conducted to compare growth performance, metal tolerance, and metal uptake by two grasses, *Thysanolaena maxima* and *Vetiveria zizanioides*. The grasses suited for phytostabilization in tropical Pb-contaminated areas of Bo Ngam Pb mine, which contain high concentrations of Pb (up 1 % total Pb) and low amounts of organic matter and major nutrients (N, P, K) (Rotkittikhun et al. 2007). Pot and field experiments showed that *T. maxima* and *V. zizanioides* had a high potential for phytostabilization Pb in mine tailing pond of KEMCO Pb mine, which ceased to operate in 2009 due to the expiry of concession. The tailing soils are poor in nutrients with high porosity to hold water but rich in Pb (Meeinkuirt et al. 2013). In pot experiment, application of pig manure increased electrical conductivity (EC) and reduced DTPA-extractable Pb concentration in the soils. The uptake by roots and transport of Pb to shoot of *T. maxima* and *V. zizanioides* were reduced when soil was amended with pig manure (Rotkittikhun et al. 2007). However, the amendment to the Pb mine soils with organic (cow manure, organic fertilizer) and inorganic fertilizer increased root biomass and Pb accumulation in the field application. Massive and complex root systems of these grasses can be pioneer plants in Pb-contaminated soil before revegetation with tree species (Meeinkuirt et al. 2013).

The potential of six tree species (*Leucaena leucocephala*, *Acacia mangium*, *Peltophorum pterocarpum*, *Pterocarpus macrocarpus*, *Lagerstroemia floribunda*, and *Eucalyptus camaldulensis*) for phytoremediation of Pb in sand tailings (total Pb >9,850 mg/kg) from the KEMCO mine were investigated employing a pot experiment (3 months) and field trial experiment (12 months). *A. mangium* with the addition of organic fertilizer was the best option for phytostabilization of Pb-contaminated mine tailing because it retained higher Pb concentration in the roots (Meeinkuirt et al. 2012). To promote woody plant *E. camaldulensis*, three Pb-tolerant bacteria (*Microbacterium paraoxydans* BN-2, *Ochrobactrum intermedium* BN-3, and *Bacillus fusiformis* BN-4) were isolated from the rhizosphere of *E. camaldulensis* grown in Pb-contaminated soils in the Bo Ngam Pb mine. Inoculation of *O. intermediu* BN-3 significantly increased the biomass and Pb accumulation by *E. camaldulensis* compared to the uninoculated control (Waranusantigul et al. 2011). A potential of growing sunflower (*Helianthus annuus*) in Pb-contaminated soil for biodiesel was tested in a pot experiment. The experiment indicated that sunflower tolerated a high Pb concentration (1,419 mg/kg) with 142 mg/kg of Pb accumulated in shoots (Sukyankij and Panichpat 2012).

6 Cadmium Contamination in Mae Sot and Reclamation

Zinc (Zn) mining was established in Mae Sot District, Tak Province, northwestern Thailand, in 1998. Paddy fields were irrigated from two creeks of Mae Tao and Mae Ku, which passed through an area where a Zn mine had been actively operated for >30 years (Pollution Control Department 2004). The Cd-contaminated areas were discovered in 12 rural villages of the district. Paddy soil samples contained Cd content above the maximum permissible level of 3.0 mg/kg during the surveys in 2001–2004 (Simmons et al. 2005). The villagers are being reported on metal toxic symptoms (Swaddiwudhipong et al. 2012). The government has supported the production of nonfood crops. Nevertheless, some residents reverted to rice cultivation mainly for their own consumption and local sale after the compensatory payment had been curtailed. Thus, it may be possible to treat rice plants with Cd-tolerant bacteria, (KKU2500-3) which could transform toxic-soluble Cd to insoluble CdS, to decrease the Cd content of rice grains and to remediate Cd-contaminated soil in the rice fields (Sripornadulsil and Siripornadulsil 2013). However, heaps of mine tailings have to be decontaminated to recover land and prevent erosion

Table 3 Potential plants for stabilization of zinc and cadmium in metalliferous soils

Chromolaena odorata	Shrub
Gynura pseudochina	Herb
Vetiveria sp.	Grass
Eucalyptus	Nonlegume tree
Leucaena leucocephala	Legume tree
Doubanga	Nonlegume tree
Ipomea aquatica	Aquatic
Pistia stratiotes	Aquatic

and leaching of Zn and Cd from the contaminated soil. The physicochemical technologies for the decontamination of contaminated ecosystems are complex and expensive and may cause undesirable side effects for the environment that then must in turn be decontaminated. Thus phytoremediation has been proposed as an effective alternative to remediate heavy metal-contaminated sites. Some potential plants for stabilization of Zn and Cd in metalliferous soils are shown in Table 3. Detailed account of phytomanagement of Padaeng Zn mine waste and Cd contamination has been dealt elsewhere (Panitlertumpai et al. 2013; Prasad et al. 2014).

7 Conclusions

Thailand has a rich phytodiversity, and hence plant and associated microflora had good potential to provide suitable phytoremediation strategies for the metalliferous substrates. The major sites contaminated with minerals in Thailand are dealt with in this chapter. Indigenous plants that can accumulate elevated doses of plants and are often associated with bacteria and fungi. However, the various processes accomplished by these associations are still not certain. A number of bio-geotech operations are deployed for reclamation of mine waste.

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Constructed Wetland: An Ecotechnology for Wastewater Treatment and Conservation of Ganga Water Quality

U.N. Rai, A.K. Upadhyay, and N.K. Singh

1 Introduction

The rapid growth of water consumption within the urban area due to industrial development, an increase of the urban population, and an improvement of sanitary conditions is accompanied by the equally rapid increase of industrial and domestic wastes polluting all the components of the natural environment: the atmosphere, water bodies, soils and subsoils.

Rapid expansion of urban areas and industrial development are often responsible for the degradation of receiving water bodies such as lakes, rivers and streams. Aquatic sediments are repositories of physical and biological debris and act as sinks for a wide variety of organic and inorganic pollutants (Zoumis et al. 2001). With substantial wastewater generation requires extensive treatment prior to environmental disposal. For the maximisation of the health and environmental benefits associated with the use and discharge of wastewater, several legislations and guidelines have been developed, both at international and national levels. Such treatment of wastewater (to discharge criteria) can be carried out using natural or conventional steel-and-concrete treatment technologies (Haberl 1999).

The Ganga basin, the largest river basin of the country, houses about 40 % of the population of India. During the course of the journey, municipal sewage, industrial effluents and wastes from several other nonpoint sources are discharged into the river Ganga resulting in its pollution. Total wastewater generation on the Ganga basin is about 8,248 MLD (CPCB 2003), out of which 2,637.7 MLD and 122 MLD municipal wastes were generated from class I and class II city situated along the river Ganga, respectively. In a report of CPCB (2010), it was estimated that only 1,117.4 MLD and 16.4 MLD municipal wastes from class I and class II cities were treated and remained without any kind of treatment. The main sources of pollution along the stretch of the river are urban liquid waste (sewage/sullage), industrial liquid waste and large-scale bathing of cattle, throwing of dead bodies in the river, surface run-off from solid waste landfills and dumpsites and surface run-off from industrial solid waste landfills. Industrial wastewater is also discharged by a number of industries situated in this riparian zone. In ecosystem terms, the joining of tributaries into the main stream gives the Ganga both pollution and power. They gain toxins and bacteria harmful to humans but also take in the water velocity and volume needed to degrade them and wash them away. Generally, scientists cite the loss of velocity as the more serious factor contributing to the rise in levels of pollution since without adequate flow, toxins and bacteria cannot be flushed and degraded.

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Over the past three decades, ecological design has been applied to an increasingly diverse range of technologies and innovative solutions for the management of resources (Todd et al. 2003). Ecological technologies are particularly considered in the field of environmental protection and restoration due to their sustainable nature. The conventional wastewater treatment systems have inherent limitations as they employ chemicals, emit foul odours, require technical skills to operate, and are expensive involving a lot of investment in construction and maintenance, and moreover they are not environmentally friendly (Carty et al. 2008). If wisdom of ecosystem be applied to the fundamental redesign of human support technologies (Todd et al. 2003), it can cogently reduce the negative human footprint on the earth up to 90 % (Hawken et al. 1999).

2 Constructed Wetland: Definition and Classification

Constructed wetlands (CWs) are engineered wastewater treatment systems that encompass a plurality of treatment modules including biological, chemical and physical processes, which are all akin to processes occurring in natural treatment wetlands. CWs have now been successfully used for environmental pollution control, through the treatment of a wide variety of wastewaters including industrial effluents, urban and agricultural storm water run-off, animal wastewaters, leachates, sludges and mine drainage (Scholz 2006) using different types of potent aquatic macrophytes and hyperaccumulator plants like bulrush (*Scirpus*), reeds (*Phragmites*), cattail (*Typha latifolia*) and knotweed (*Polygonum* sp.), lotus (*Nelumbo nucifera*), nilofar (*Nymphaea alba*), *Ceratophyllum demersum*, etc.

There are two basic types of constructed wetlands: surface flow and sub-surface flow systems (Kadlec and Knight 1996).

2.1 Surface Flow (SF)

Surface flow wetlands are similar to natural wetlands, with shallow flow of wastewater (usually less than 60 cm deep) over saturated soil substrate. The use of SF systems is extensive in North America. These systems are used mainly for municipal wastewater treatment with large wastewater flows for nutrient polishing. The SF system tends to be rather large in size with only a few smaller systems in use.

The majority of constructed wetland treatment systems are surface flow or free water surface (SF) systems. These types utilise influent waters that flow across a basin or a channel that supports a variety of vegetation, and water is visible at a relatively shallow depth above the surface of the substrate materials. Substrates are generally native soils and clay or impervious geotechnical materials that prevent seepage (Reed et al. 1995). Inlet devices are installed to maximise sheet flow of wastewater through the wetland to the outflow channel. Typically, bed depth is about 0.4 m.

2.2 Sub-Surface Flow (SSF) System

Sub-surface flow wetlands mostly employ gravel as the main media to support the growth of plants; wastewater flows vertically or horizontally through the substrate where it comes into contact with microorganisms, living on the surfaces of plant roots and substrate (Cooper et al. 1996; Kadlec and Knight 1996), allowing pollutant removal from the bulk liquid. The SSF system includes soil-based technology which is predominantly used in Northern Europe, and the vegetated gravel beds are found in Europe, Australia, South Africa and almost all over the world.

Sub-surface flow constructed wetlands are further divided into two groups:

1. Vertical flow (VF)
2. Horizontal flow (HF)

Much interest has developed in recent years to study the mechanism and possible impact of wetlands to remove contaminants from water, whether it is effluent from municipal or private waste systems and industrial or agricultural wastewater. A constructed wetland uses natural geochemical and biological processes in a wetland ecosystem to treat metals, explosives and other contaminants in groundwater.

3 Removal Mechanism in Constructed Wetland

A constructed wetlands treatment system (CWTS) is a bed composed of the substrate, water-tolerant plants, the water column and a microbial population. The substrate can be sand, gravel or soil in which wetland plants are growing. Natural wetlands act as biofilter, removing sediments and pollutants such as heavy metals from the water. Vegetation in a wetland provides a substrate (roots, stems and leaves) upon which microorganisms can grow as they break down organic materials. There are more indirect ways in which plants contribute to wastewater purification. Plants create a unique environment at the attachment surface of the biofilm.

Treatment of wastewater within a constructed wetland occurs as it passes through the wetland medium and the plant rhizosphere. Decomposition of organic matter is facilitated by aerobic and anaerobic microorganisms present. Microbial nitrification and subsequent denitrification release nitrogen as gas to the atmosphere. The removal of nitrogen from wastewater is important because of toxicity of ammonia to fish if discharged into water courses.

3.1 Role of Plants and Microorganisms in Constructed Wetland

Plants play an important role in CO_2 mitigation in the atmosphere, settlement of suspended particulate matter (SPM) on their leaf and minimisation of the flow of toxic metals in sewage/contaminated water flowed in the river stream. Heavy metals such as cadmium, zinc, nickel, lead, chromium and copper are concentrated in the river water and the sediments. Besides, nitrate and phosphates are also found in toxic levels. Certain plants transport oxygen which is released at the biofilm/root interface perhaps adding oxygen to the wetland system (Pride et al. 1990). Plants also increase soil or other root-bed medium hydraulic conductivity. As roots and rhizomes grow, they are thought to disturb and loosen the medium increasing its porosity which may allow more effective fluid movement in the rhizosphere. When roots decay, they leave behind ports and channels known as macropores which are effective in channelling water through the soil. Higher plants in wetland systems may be viewed as transient nutrient storage compartments absorbing nutrients during the growing season and releasing large amounts at senescence. Aquatic vegetation may play an important role in phosphorus removal and, if harvested, extend the life of a system by postponing phosphorus saturation of the sediments. Different species of aquatic plants have different rates of heavy metal uptake; a consideration for plant selection in a constructed wetland used for water treatment and plants such as cattails (*Typha* sp.), sedges, water hyacinth (*Eichhornia crassipes*) and *Pontederia* sp. are used worldwide; however, for subarctic regions, buckbeans (*Menyanthes trifoliata*) and pendant grass (*Arctophila fulva*) are also useful for metal uptake.

Bacteria are of the greatest numerical importance in the constructed wetland system. The preponderance of bacteria living is facultative, and they are able to live in either the presence or absence of oxygen (Spellman 1997; Absar 2005). Although both heterotrophic and autotrophic bacteria are found in constructed wetland but, the former predominate and obtain energy from the carbonaceous organic matter in the influent wastewater for the synthesis of new cells and also release energy via the conversion of organic matter compounds such as CO_2 and H_2O. Important heterotrophic bacteria in activated sludge are *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Citromonas*, *Flavobacterium*, *Pseudomonas*, *Zoogloea* and *Acinetobacter* (Oehmen et al. 2007). Many of these bacteria form floc particles or clusters of bacteria that break down waste. When filamentous bacteria are present in excessive numbers or length, they often cause solid/liquid separation or settleability problems (Gray 2002; Paillard et al. 2005).

A small number of fungi are capable of oxidising ammonia to nitrite and fewer still to nitrate. The most common sewage fungus organisms are *Sphaerotilus natans* and *Zoogloea* sp (Painter 1970; LeChevallier and Au 2004). Besides, some

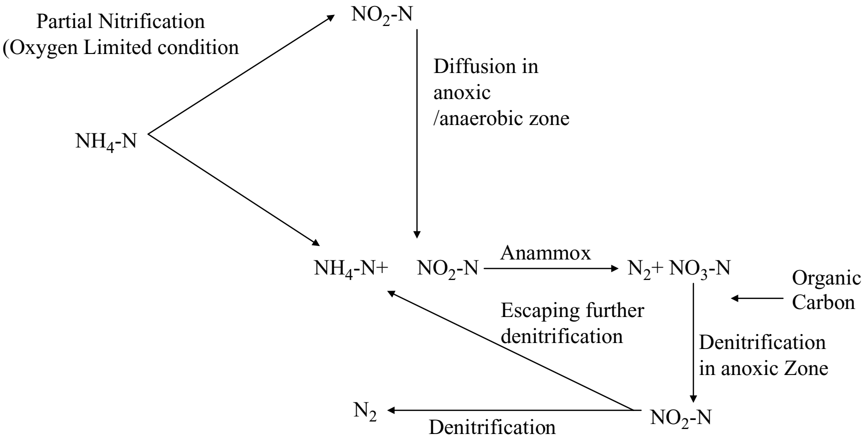

Fig. 1 Conceptual diagram of simultaneous nitrogen removal via Anammox process and denitrification in sub-surface flow wetland (Saeed and Sun 2012)

members of Basidiomycotina, Ascomycotina and Deuteromycotina are important phosphate-solubilising microbes. *Penicillium* sp., *Aspergillus niger*, *A. flavus*, *A. fruticans*, *Fusarium* sp., *Rhizopus nigricans* and *Alternaria* sp. are important fungi used in bioremediation processes.

3.2 Removal of Nitrogen

Besides conventional nitrogen removal, newly discovered nitrogen removal routes are solely dependent on microbiological metabolism such as partial nitrification, denitrification, Anammox (anaerobic ammonium oxidation: anaerobic oxidation of ammonium directly to nitrogen gas by nitrite in the presence of planctomycete bacteria group) and Canon processes (completely autotrophic nitrite removal over nitrate). Nitrogen removal through partial nitrification and denitrification process includes conversion of NH_4-N to NO_2-N, followed by denitrification of NO_2-N to N_2 gas (Fig. 1) (Saeed and Sun 2012).

$$NH4^{+} + 1.5O_2 \rightarrow NO_2^{-} + H_2O + 2H^{+}$$

$$NO_2^{-} + 1/2CH_3OH + H^{+} \rightarrow 1/2N_2 + 1/2CO_2 + 1.5H_2O$$

The advantages of partial nitrification and denitrification include approximately 25 % lower oxygen and 40 % lower organic concentration requirements, compared with traditional nitrification and denitrification metabolism (Jianlong and Ning 2004).

3.3 Removal of Organic Content

Organic matter is decomposed in constructed wetlands by both aerobic and anaerobic microbial processes as well as by sedimentation and filtration of particulate organic matter. Organic matter is broken down by microorganisms colonising the wetland by fermentation and aerobic or anaerobic respiration and mineralised as a source of energy or assimilated into biomass. Other removal mechanisms include sedimentation, sorption and volatilisation. Aerobic degradation of soluble organic matter is governed by the aerobic heterotrophic bacteria according to the following reaction:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 12e - + \text{energy}$$

In most types of wastewaters with the exception of some industrial wastewaters and run-off waters, the supply of dissolved organic matter is sufficient, and aerobic degradation is limited by dissolved oxygen concentration (Vymazal 2001). Organic matter is composed of a complex mixture of biopolymers (Megonigal et al. 2004). Some of these compounds, such as proteins, carbohydrates and lipids, are easily degraded by microorganisms (i.e. labile), while other compounds, such as

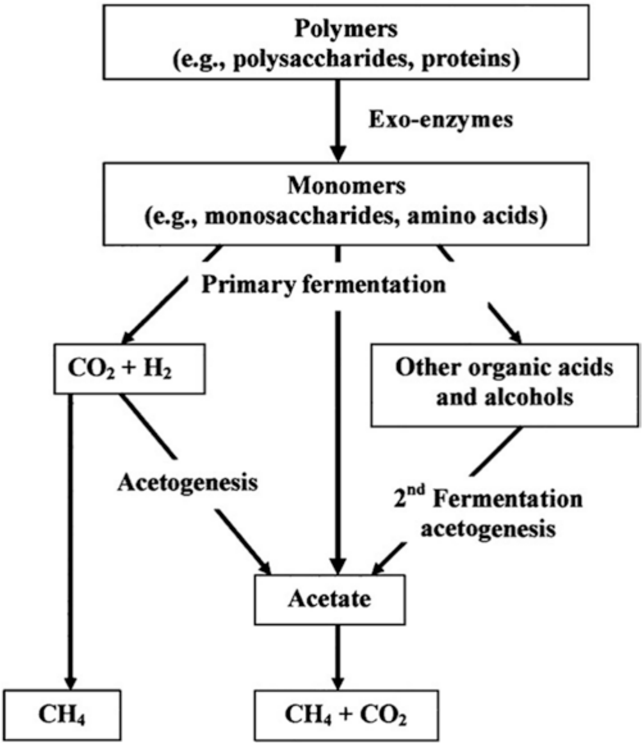

Fig. 2 Metabolic scheme for the degradation of complex organic matter, culminating in methanogenesis (Megonigal et al. 2004)

lignin and hemicellulose, are resistant to decomposition (i.e. recalcitrant). Biopolymers are degraded in a multistep process (Fig. 2).

3.4 Phosphorus Removal

Based on the phosphorus cycle, the only means of removing phosphorus from wastewater by a constructed wetland is through accumulation of phosphorus within the system. This accumulation of phosphorus can occur physically, biologically or chemically.

3.4.1 Physical Removal Mechanism

Physical processes function by removing phosphorus in suspended solids and in precipitates by filtration, with subsequent sedimentation in the substrate media within the wetland. Suspended particles in the influent may combine with a number of soluble and insoluble phosphorus forms in the wetland. However, if the solid matter filtered from the wastewater is planktonic, its decomposition can release dissolved phosphorus into the water column (Kadlec and Knight 1996). Filtration is primarily mechanical straining where the particles larger than the pore size of the strainer (filtering medium) are separated from the bulk of the liquid. Sedimentation is separation of suspended particles that are heavier than water by gravitational settling (Metcalf and Eddy 2003).

3.4.2 Biological Removal Mechanisms

Biological storage of phosphorus occurs through uptake by plants and microbes; though this quantity maybe small. During the start-up phase, phosphorus is taken up by biomass as it becomes established (Mander and Jenssen 2002). However, as wetlands mature to steady state, there is little net removal through uptake. By carefully managing the growing and harvesting of vegetation, bioretention of phosphorus can be controlled (Davies et al. 2006). Cooke (1992) found that 11 % of the phosphorus in the wetland was taken up by the plants. Another study found that uptake by cattails was 40 % of the total phosphorus input (Weng et al. 2006).

In a constructed wetland system receiving secondarily treated domestic wastes contained 40.5 % of the total phosphorus influent. The remaining 59.0 % was found to be stored in the gravel substratum. Phosphorus removal in a surface flow wetland treatment system planted with one of *Scirpus* sp., *Phragmites* sp. or *Typha* sp. was investigated by Finlayson and Chick (1983). Most phosphorus transformations mediated by microorganisms involve the mineralisation of organic to inorganic phosphates (a process also referred to as "decomposition") or the conversion of insoluble, immobilised forms of tertiary phosphate into soluble, mobile primary phosphates that are more readily used by organisms. Mineralisation of organic to inorganic phosphate involves processes catalysed by phosphatase enzymes, which are specifically involved in this conversion. Many microorganisms produce these enzymes. Phosphatase enzymes are present in all organisms, but only bacteria, fungi and some algae are able to secrete them outside of their cells. As exoenzymes, they participate in the dissolution and mineralisation of organic phosphate compounds in the environment. Without phosphatase enzymes, the presence of inorganic phosphorus would be limited to external sources, such as fertilisers, and primary productivity would be limited and dependent on these external sources. Phosphate would remain sequestered in cell matter and unavailable for producers. The enzymatic activity of microbial communities is critical for the proper cycling of phosphorus within an ecosystem.

3.4.3 Phosphorus Solubilisation

Plant phosphorus nutrition has always been of great concern as it is the most limiting nutrient. About 96 % of phosphorus is present in the soil as insoluble phosphate; inorganic phosphorus is locked into crystal lattices of clay particles and is the main reason behind its low solubility. Thus, soils worldwide are deficient in assimilable inorganic phosphate (H_2PO_4) form. This low solubility of phosphatic compounds which is a characteristic feature of soil phosphorus is responsible for its limiting concentration in soil solution and hence its unavailability to plants. Rock phosphate cannot be directly utilised to replenish phosphorus requirement of plants but instead utilised in the manufacture of superphosphate fertilisers by employing very costly and tedious processes. Phosphorus promotes development of deeper roots. To increase the availability of phosphorus for plants, large amounts of fertiliser is used on a regular basis. But after application, a large proportion of fertiliser phosphorus is quickly transferred to the insoluble forms.

Phosphorus has an important role to play in plant growth and nutrition. It increases biological activities like nodulation, nitrogen fixation and nutrient uptake in soil and rhizosphere environment resulting in higher yield of crops. Its deficiency results in stunted growth, lack of chlorophyll, deepening of green and red colour of leaves, and abnormal/arrested root development and maturity delay of plant.

In this regard, some microorganisms play an important function of solubilising phosphatic compounds and making them available for plant assimilation. Phosphorus solubilisation is carried out by a large number of saprophytic bacteria and fungi acting on sparingly soluble soil phosphates, mainly by chelation-mediated mechanisms (Whitelaw 2000). Some fungal species have mineralisation and solubilisation potential for organic and inorganic phosphorus, respectively (Hilda and Fraga 2000). Phosphorus-solubilising activity is determined by the ability of microbes to release metabolites such as organic acids, which, through their hydroxyl and carboxyl groups, chelate the cation bound to phosphate, the latter being converted to soluble forms (Sagoe et al. 1998). Phosphate solubilisation takes place through various microbial processes/mechanisms including organic acid production and proton extrusion (Dutton and Evans 1996; Nahas 1996).

3.4.4 Chemical Removal Mechanisms

Phosphorus removal through chemical processes is the most important means of accumulating phosphorus in constructed wetlands (Kadlec and Knight 1996). Chemical removal of phosphorus occurs through precipitation and sorption processes.

3.4.4.1 Precipitation

Precipitation is the formation of insoluble products from the combination of soluble reactants. The removal of phosphorus is ultimately achieved through physical storage of the phosphorus precipitates within the substrate media. Naturally occurring soluble iron and aluminium or calcium can result in significant removal of phosphorus by precipitation (Ayoub et al. 2001; de-Bashan and Bashan 2004). To enhance the phosphorus precipitation within a wetland, at times, chemical dosing is required, although this chemical dosing can be a great disadvantage as the operational simplicity inherent in the wetland system is lost and the potential for clogging increases (Brady and Weil 1999).

Alum [$Al(SO_4)_2.12H_20$] and ferric chloride ($FeCl_3$) are most commonly used in conventional wastewater treatment facilities to precipitate phosphorus, with subsequent physical removal from the system by co-settling with the organic suspended solids (Lind 1998).

$$Al_2(SO_4)_3 + 2Na_3PO_4 \rightarrow 2AlPO_4 \downarrow + 3Na_2(SO_4)$$

$$Al_2(SO_4)_3 + 6H_2O \rightarrow 2Al(OH)_3 \downarrow + 3H_2SO_4$$

$$FeCl_3 + Na_3PO_4 \rightarrow FePO_4 \downarrow + 3NaCl$$

$$FeCl_3 + 3H_2O \rightarrow Fe(OH)_3 \downarrow + 3HCl$$

Lime (CaO) can also be used for phosphorus removal as (Lind 1998):

$$3CaO + 2Na_3PO_4 + 3H_2O \rightarrow Ca_3(PO_4)_2 \downarrow + 6NaOH$$

$$CaO + H_2O \rightarrow Ca(OH)_2 \downarrow$$

$$CaO + CO_2 \rightarrow CaCO_3 \downarrow$$

Chemical dosing is usually done prior to the wetland. The operation and implementation of chemical dosing is simple but results in increased sludge and associated disposal costs and increased operation and maintenance costs. The quality of effluent achieved can be as low as 0.1 mg/L of phosphorus using chemical dosing.

3.4.4.2 Sorption

Sorption is the process of a substance adhering to the surface of a wetland media and is considered to be the most significant phosphorus removal mechanism (Moshiri 1993). Sorption is a general term that encompasses both adsorption and absorption mechanisms and is a time-limited process based on the available surface area. Adsorption is the process of accumulating substances on the media surface, while absorption refers to the accumulation within the pore structure of the media (Stumm and Morgan 1996). Sorption reactions are based on an equilibrium condition where the rates of reaction are fast in relation to the physical processes of advection and dispersion (Kueper 2003). The process occurs on two different levels, viz., molecular and filter bed scales.

4 Removal of Heavy Metals

A heavy metal is a member of a loosely defined subset of elements that exhibit metallic properties. It mainly includes the transition metals, some metalloids, lanthanides and actinides. When heavy metals enter a CW, they are distributed among its compartments with the main pools being the substrate, the water column and the vegetation (Dunbabin and Bowmer 1992; Sheoran and Sheoran 2006). Kadlec and Knight (1996) described the following major processes responsible for the removal of heavy metals in CWs: (i) binding to the substrate, particulates and soluble organics; (ii) precipitation as insoluble salts, mainly sulphides and (oxy)hydroxides; and (iii) uptake by plants and microorganisms. There are only minor gaseous removal pathways for some metals including Hg, Se and As. Sheoran and Sheoran (2006) have subdivided the mechanisms into physical, chemical and biological processes although the authors emphasised that the processes are dependent on each other.

4.1 Physico-chemical Removal Mechanisms of Heavy Metals

Major physico-chemical removal processes of heavy metals occurring in CWs include (1) sedimentation and filtration, (2) sorption and (3) precipitation and co-precipitation.

4.1.1 Sedimentation and Filtration

Sedimentation and filtration are important physical processes allowing the removal of heavy metals associated with particulate matter. Sedimentation has long been recognised as a principle process in the removal of heavy metals from wastewaters (Sheoran and Sheoran 2006).

4.1.2 Sorption

Sorption of heavy metals can occur by processes of adsorption and precipitation. Heavy metals may be adsorbed either electrostatically, resulting in the formation of relatively weak complexes (physical adsorption), or chemically, resulting in the formation of strong complexes (chemisorption). Metals are more strongly bound by chemisorption than by physical adsorption (Evangelou 1998).

4.1.3 Precipitation and Co-precipitation

Metals can also precipitate with (oxy)hydroxides, sulphides, carbonates, etc. when solubility products are exceeded (Kadlec and Knight 1996). The stability of metals precipitated as inorganic compounds is primarily controlled by pH. At near-neutral or alkaline pH, metals are effectively immobilised (Gambrell 1994). Bacterial production of bicarbonate by bacterial sulphate reduction, or the presence of limestone in the substrate, can lead to sufficiently high bicarbonate levels to form precipitates with metals. Hydrolysis and/or oxidation of metals leads to the formation of (oxy)hydroxides. The solubility of these oxides is very low in the range of pH normally encountered in most substrates (Evangelou 1998). Dissolved reduced forms of Fe (II) are oxidised by abiotic reactions and bacteria and then precipitated mainly as hydroxides, a reaction which increases the acidity of the wastewater (Kosolapov et al. 2004):

$$\mathrm{Fe^{2+} + H^{+} + 41O2 \rightarrow Fe^{3+} + 21H2O}$$

$$\mathrm{Fe^{3+} + 3H2O \rightarrow Fe(OH)3 \downarrow + 3H^{+}}$$

After the oxidation of Fe (II), Mn (II) is oxidised to Mn (IV) which is mainly precipitated as MnO2 but can also be precipitated as Mn(OH)2. Iron and Mn precipitate sequentially because the oxidation of Mn is inhibited by the presence of Fe (II) (Kosolapov et al. 2004). Co-precipitation of metals with Fe and/or Mn (oxy) hydroxides is an additional important removal pathway in oxidised substrates. Co-precipitation with Fe and/or Mn (oxy) hydroxides is however not considered as a long-term removal mechanism as it is redox sensitive (Sheoran and Sheoran 2006).

In anaerobic conditions and the presence of an organic carbon source, sulphate is reduced by sulphate-reducing bacteria (SRB), and sulphides are formed. Sulphides then react with divalent metals to form insoluble metal sulphide precipitates (Gambrell 1994; Kosolapov et al. 2004):

$$\mathrm{SO4^{2-} + 2CH2O \rightarrow H^{+} + HS^{-} + 2HCO3^{-}}$$

$$\mathrm{H^{+} + HS^{-} \rightarrow H2S(g)}$$

$$\mathrm{HS^{-} + M^{2+} \rightarrow MS \downarrow + H^{+}}$$

With CH2O and M^{2+} representing, respectively, a simple organic compound and divalent metal ion. The process of sulphate reduction buffers the pH of the solution.

4.2 Biological Removal Mechanisms of Heavy Metals

4.2.1 Microbial Removal Processes

Microorganisms affect the behaviour of heavy metals in constructed wetlands by (i) their role in the biogeochemical cycles which affect metal speciation, (ii) biosorption, (iii) reduction and (iv) methylation of heavy metals (Kosolapov et al. 2004).

4.2.1.1 Biosorption of Heavy Metals

The microbial biomass can also sequester metals by the processes of active (energy-dependent) and passive (energy-independent) metal uptake, respectively, called bioaccumulation and biosorption (Kosolapov et al. 2004). Some bacteria are also able to accumulate metals inside their cells, forming amorphous mineral inclusions. However, the storage of metals by the microbial biomass is relatively short-term due to the short life cycle of microorganisms implying the need for additional removal pathways (Kosolapov et al. 2004).

4.2.1.2 Reduction of Heavy Metals

Anaerobic metal-reducing bacteria use metals as terminal electron acceptors in their anaerobic respiration. Depending on the metal species involved, this can lead to a mobilisation or immobilisation of metals. The reduction of the soluble and toxic Cr (VI) to Cr (III) results in its precipitation with hydroxides and efficient removal from wastewater. Reduction of Cr (VI) is performed by a variety of microorganisms, including some sulphate-reducing bacteria (SRB), and can occur under oxic and anoxic conditions. Whereas the reduction of Cr (VI) leads to its effective immobilisation and retention in the substrates of CWs, reduction of other metals including Hg, Fe and Mn enhances their mobility and releases from the wetland system. The reduction of Hg (II) results in the release of elemental Hg to the atmosphere. This strategy has been successfully applied to remediate Minamata Bay sediments. However, there are public concerns about atmospheric pollution. Iron- and Mn-reducing bacteria can dissolve insoluble Fe (III) and Mn (IV) (oxy)hydroxides, resulting in the release of soluble Fe (II) and Mn (II), as well as the trace metals co-precipitated with these (oxy) hydroxides. Intermediate redox conditions are detrimental to CW efficiency as they transfer metals to their most soluble state (Kosolapov et al. 2004).

4.2.1.3 Methylation of Heavy Metals

Some metals, including Hg, As and Se, can be biomethylated by aerobic and anaerobic bacteria resulting in the production of volatile derivatives such as dimethylmercury, dimethylselenide or trimethylarsine (Salt et al. 1995; Kosolapov et al. 2004). These volatile derivatives are generally more toxic than the inorganic metal species, because of their lipophilic character. Another toxic product of the methylation of Hg is methylmercury (CH3Hg), a lipophilic product that is easily accumulated by aquatic organisms and transferred into the food chain. Some SRB (sulphate-reducing bacteria) have been identified to methylate Hg, implying the need for control when the process of sulphate reduction is aimed to treat wastewaters that are also contaminated by Hg. However, the precipitation of HgS has been described to reduce Hg toxicity (Kosolapov et al. 2004).

4.3 Uptake of Heavy Metals by Aquatic Macrophytes

Uptake of heavy metals by aquatic macrophytes is dependent on the life form of the macrophyte: rooted emergent, floating or submerged (Guilizzoni 1991). For emergent macrophytes, root uptake is the primary source of metals. Floating and submerged species, however, can also accumulate metals directly from the water by their shoots. Elucidation of a single metal uptake pathway is difficult without the use of tracers, and a comparison of several uptake patterns simultaneously is even more complex (Crowder 1991).

Aquatic macrophytes depend on the micronutrients available in the water and/or substrate, in order to meet their nutritional demands. Among the micronutrients that are required for the growth and development of all higher plants, the following heavy metals are encountered: Fe, Mn, Zn, Cu, Mo and Ni (Welch 1995). Certain plants can also accumulate metals with no known biological function, including Cd, Cr, Pb, Co, Ag, Se and Hg (Salt et al. 1995).

4.3.1 Uptake of Heavy Metals by the Roots

Most metal ions enter plant cells by specific metal ion carriers or channels. After entering the roots, metals are either stored or transported to the shoot. Transport to the shoot probably occurs in the xylem, and metals can be redistributed in the shoot via the phloem. Plants take up elements in their ionic form. Aquatic macrophytes which root in the substrate rely on the soluble metal fraction which is affected by parameters including pH, redox potential and organic matter content of the substrate (Jackson 1998). Plant roots can mobilise metals by various strategies, including (i) the excretion of metal-chelating molecules (phytosiderophores) that solubilise metals, (ii) the plasma membrane-bound metal reductases that reduce metal ions and (iii) the excretion of protons (Salt et al. 1995; Meers 2005). Solubilised metal ions may enter the roots by extracellular (apoplastic) or intracellular (symplastic) pathways. To enter the xylem, metals must cross the Casparian strip that divides the endodermis from the epidermis. Metals must move symplastically to cross this strip. The symplastic transport within the endodermis is a rate-limiting step. Xylem cell walls have a high cation exchange capacity which would severely retard the movement of metal cations to the shoot. Metals in the xylem and phloem may be transported chelated to organic acids, phytochelatins or metallothioneins (Raven et al. 1992; Salt et al. 1995).

Non-linear removal kinetics of heavy metals have been reported, suggesting that rooted aquatic macrophytes use several removal mechanisms. Sorption by the root is probably the fastest component of metal removal including processes such as physical adsorption and chemisorption (chelation and ion exchange). Biological processes including intracellular uptake and translocation to the shoots and root-mediated precipitation are probably responsible for the slower components of metal removal (Salt et al. 1995; Maine et al. 2004). Studies in many natural and constructed wetlands have demonstrated similar allocation patterns of metals in rooted emergent macrophyte species, with most plants having higher concentrations of metals in their belowground biomass than in their shoot tissues (Weis and Weis 2004). Restricted translocation of metals to the shoots is at the basis of this finding.

4.3.2 Uptake of Heavy Metals by the Shoots

For floating or submerged macrophytes, plants of which the shoots are in close contact with the water or are completely inundated, roots are not the sole entry points of metals. According to Guilizzoni (1991) and Keskinkan (2005), there is scarce study about heavy metal uptake by submerged and floating aquatic plants, in particular about the relative importance of shoot/root uptake. Roots of submerged aquatic macrophytes were initially thought to act as holdfasts, whereas element uptake was mainly through the leaves. This was suggested by early studies which showed a good correlation between metal concentrations in the leaves and the surrounding water. For aquatic macrophytes that have roots but do not have a close association with the sediment, the water is probably the principal source of elements. However, the situation for submerged macrophytes with a well-developed root–rhizome system and submerged foliage such as *Myriophyllum* and *Potamogeton* is much more complex (Guilizzoni 1991). Studies with radioactive tracers showed that elements are mainly taken up by the roots from the sediment (Jackson 1998). Guilizzoni (1991) hypothesised that the water is the dominant source of elements when dissolved metal concentrations in the water are high or when metals are not readily available in the sediment. This hypothesis was supported by Maine et al. (2004) who studied the causes of the increase of the Cr concentration in the shoots of the floating macrophytes *Pistia stratiotes* and *Salvinia herzogii*, when exposed to Cr-contaminated solutions. In an attempt to address whether (i) translocation from the roots to the shoots or (ii) direct uptake by the shoots through contact with the solution was the responsible mechanism, the experimental set-up included plants of which the aerial parts were exposed to the water and plants of which the aerial parts were separated from the water by means of thin sheets of polystyrene. The increase of the Cr concentration in the shoots was mainly attributed to the direct contact between the shoots and the water and not by translocation. Guilizzoni (1991) suggested that the uptake of ions by submerged leaves involves a binding step to the cell membrane and a transfer inside. Cations enter the abaxial epidermis by means of a multiple mechanism of passive (diffusion) or active uptake. After uptake, metals are translocated in different plant parts. It still remains unclear what factors govern root or shoot absorption and by what mechanisms the translocation occurs.

5 Green Technologies for Ganga Water Rehabilitation

5.1 Current Initiatives for Clean Ganga

Now, the Centre has set up the National Ganga River Basin Authority (NGRBA) on 20 February 2009 under the Environment Protection Act as an empowered authority to adopt a new holistic river basin approach to the cleaning of the river Ganga and address the issue of minimum ecological flows, besides pollution abatement works comprehensive management of the Ganga river basin under the chairmanship of the Prime Minister. This body will see that development requirements (such as construction of hydropower projects) are met in a sustainable manner while ensuring ecological flows. The body will not be a separate additional clearance mechanism. Rather, it will develop a management plan for the river basin and address pollution abatement measures by ensuring adequate ecological flow in the river. Specific interventions for sewage treatment have also been planned.

In last few years, CSIR-National Botanical Research Institute has undertaken a research programme on “Plant based management of Ganga water pollution” sponsored by the National River Conservation Directorate, Ministry of Environment & Forests, New Delhi. In order to rehabilitate the river Ganga ecosystem, a sub-surface flow constructed wetland has been developed at Shantikunj, Haridwar, using efficient aquatic macrophytes, *Typha latifolia*, *Phragmites australis*, *Colocasia esculenta*, *Polygonum hydropiper*, *Alternanthera sessilis* and *Pistia stratiotes* with gravel as medium, and its sewage treatability potential was evaluated. All the plants grew well in the gravel media without any symptom of toxicity or nutrient deficiency. After 3 months of plantation, plants were found established and showed luxuriant growth during 6 months of operation and monitoring of wetland (Fig. 3).

5.2 A Case Study

Monitoring results at 6 months of operation of wetland showed improvement of sewage water quality as reduction rate of BOD, TSS, TDS, NO_3–N, PO_4–P and NH_3–N was found to increase with growth and establishment of the plants and found maximum in fully established constructed wetland as compared to pre- and partial established after 2 and 4 months. Further, DO level was increased from 2.67 to 4.71 mgL^{-1} in pre- and fully established constructed wetland after 24 h of retention time, respectively. Similarly, sewage treatment efficiency of constructed wetland increased from pre-established stage (after 2 months) to fully established stage (after 6 months) in terms of BOD and TSS which increased from 18–90 % to 44–65 %, respectively, after 24 h of treatment. Removal values of

Settling Tank A View of SSF constructed wetland Outlet drainage of treated water

Fig. 3 Sub-surface flow constructed wetland at Shantikunj, Haridwar

NO_3–N and PO_4–P were also increased from 65–84 % to 11–76 % in pre-established stage (after 2 months) and fully established stage (after 6 months), respectively. Constructed wetland was found to remove more than 90 %, 65 %, 78 %, 84 %, 76 % and 86 % of BOD, TSS, TDS, NO_3–N, PO_4–P and NH_3–N, respectively, from inlet sewage after 36 h of retention time under fully established condition. Therefore, vegetation in a wetland provides a substrate (roots, stems and leaves) upon which microorganisms can grow as they break down organic materials. There are more indirect ways in which plants contribute to wastewater purification. Further, treatment through fully established constructed wetland resulted in reduction in metal contents in sewage water, including Cr, Mn, Co, Ni, Cu, Zn, As, Se, Cd and Pb.

6 Utilisation of Phytoremediated Biomass

In most of the composting processes, the focus is on waste management and mass reduction. Phytoremediated biomass of aquatic plant contains high amount of toxic metals, which has important role in biogeocycling via active and passive transfer of elements into the food chain. To overcome this problem, composting has been found to be one of the most economical ways of treatment because it combines materials recycling and waste disposal at the same time. Several wetland macrophytes such as *Spirodela polyrrhiza*, *Ceratophyllum demersum*, *Bacopa monnieri*, *Alternanthera sessilis*, *Hygroryza aristata*, *Hydrilla verticillata* and *Vallisneria spiralis* have been utilised for postharvest composting. Besides, key factors for a successful composting such as temperature, aeration, moisture and nutrients should be appropriately controlled. C/N and C/P ratio is one of the important factors effecting composting process and compost quality (Huang et al. 2004). Among the wetland plants, *V. spiralis* has been found as a potential plant for water quality improvement, reduction of metal and composting process having high C/N ratio (Shukla et al. 2009).

Public perception of composting tends to be positive insofar as there is a general acceptance of the need to recycle or reduce waste, but there are environmental issues which here become driver or constraints, subsequent to the process – rate/cost equation. The biogas plant is the perfect fertiliser-making machine, and it has been tested all over the world. There is no better way to digest or compost manure and other organic material than in a biogas plant. Bio-compost is a 100 % natural and organic fertiliser, thereby also aids in the better establishment of plants. Bio-compost is a cost-effective and eco-friendly supplement to chemical fertilisers.

7 Conclusions

Continuous rise in the pollution of river Ganga has been accompanied by mass apathy. Pollution and public concern of Ganga seem to exist in inverse ratios. The distressed river beckons all to come to its rescue. Implementation of plant-based green technology of constructed wetland for sewage treatment before mixing into Ganga river at its source and popularisation in local public residing along the bank of Ganga river by awareness among masses about pollution threats in the river has to go hand in hand. A study conducted in Shantikunj, Haridwar, India, showed that the plants developed in fully established constructed wetland have greater purifying potential for sewage from domestic sources as concentration of different physico-chemical parameters was reduced to an acceptable limit for discharging into the river Ganga. Therefore, there is a need of a new vision for a pristine and pure Ganga to pour forth and translate on the ground. A new vision, which needs churning of the spirit and mind, inspires the masses to action and reconcile the competing demands on the precious waters of the river with sustainability. It needs to think of the river as one organic entity where tinkering in one part affects the entire body of the river. Use of the constructed wetlands for filtration and remediation of water is currently a popular method; however, understanding the nature of plant–microbe interactions may improve this process.

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Mycorrhizal Plants' Accelerated Revegetation on Coal Mine Overburden in the Dry Steppes of Kazakhstan

D.V. Veselkin, A.N. Kupriynanov, Ju.A. Manakov, A.A. Betekhtina, and M.N.V. Prasad

Abstract

Coal mine (Fedorovsky open-pit mine) overburden of Karaganda, Kazakhstan, is characterized by plants associated with mycorrhizae. This is a long-term work that was carried out methodically in 1978, 1980, 1990, and 2006 in different parts of overburden rocks of Karaganda, Kazakhstan. During the process of development of vegetation on the overburden rocks, there has been an increase in the composition of obligate species associated with arbuscular mycorrhizal fungi. The proportion of non-mycorrhizal species declined gradually. Phytosociologic investigation of these plant communities over a range of period made it possible to estimate the rate of changes in the ratio of plants of different mycorrhizal groups. Despite the fact that the total number and species composition gradually changed, different mycorrhizal species stabilized after the early succession (5 years). The ecto- and endomycorrhizal fungi enhanced growth of the three woody species on these overburden sites.

Keywords

Mycorrhizas • Herbs • Mycorrhizal • Non-mycorrhizal • Early succession • Vegetation dynamics

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1 Introduction

Due to emerging economies, consumption of coal is increasing largely all over the world. Worldwide, 40 % of electricity generation is based on coal-based super thermal power plants. Coal is effectively exploited not only in Russia, but also in other parts of the world. The environmental impacts of coal mine overburden, pollution monitoring of abandoned coal mines, and reclamation are some of the emerging areas of research. Coal mine overdump leachates will adversely affect the biodiversity and environment (Bian et al. 2009). Further, large tracts of land are required to dispose of coal mine waste which ultimately pollutes the environment (Ghose and Majee 2000). All these negative impacts of mining waste can have long-lasting environmental and socioeconomic consequences, and restoration is a subject of environmental concern (Brenner 1984; Glazyrina et al. 2007; Glebova 1992; Weng et al. 2012). Coal mine spoils and overdumps, therefore, have to be properly managed to ensure the long-term stability of disposal facilities and to prevent or minimize air, water, and soil pollution (Anonymous 1998; Bell et al. 2001; Bian et al. 2009; Dutta and Agrawal 2001; Fresquez et al. 1987; Ghose and Majee 2000; Vorobeychik et al. 1994; Xu et al. 2005). Due to low water retention and low fertility, coal mine overdumps do not support plant growth. However, mycotrophy is a beneficial biotic interaction and improves soil structure and promotes plant growth. Mycorrhizal fungi are the main component of the soil microbiota in most ecosystems, and a majority of the terrestrial plants are associated with some kind of mycorrhizal fungi, arbuscular mycorrhizal fungi (AMF) being the most common group (Betekhtina and Veselkin 2011; Call and Davies 1988; Chibrik et al. 1980; Chibrik and Salamatova 1985). In this symbiotic association, host plants provide the fungi with carbohydrates and in return receive mineral nutrients.

In the last few decades, there has been a great surge and progress in studying the role of mycorrhizal symbiosis in the processes of vegetation dynamics (Ahulu et al. 2005; Bannari et al. 1995; Gemma and Koske 1990; Han et al. 2007), postglacial (Jampponen et al. 2002), coastal (Püschel et al. 2007a), and others successions (Miller 1979; Pezzani et al. 2006). These investigations have established that the mycorrhizal incidence fostered the nutrient acquisition in plants establishing vegetation succession on nutrient-poor substrates (Lambers et al. 2008; Heijden et al. 1998, 2003; Pezzani et al. 2006; Püschel et al. 2007b).

It is of undoubted interest to compare the patterns obtained in natural conditions with the paterns, which manifest themselves in artificially (technological) formed habitats, characterized often by extreme or adverse physical and chemical properties of substrates (soils). The significance of mycorrhizal research in man-made habitats in particular on the overburden of different mines is not new (Chibrik et al. 1980; Chibrik and Salamatova 1985; Cornelissen et al. 2001; Cuenca et al. 1998; Daft and Nicolson 1974; Davies and Call 1990; Dutta and Agrawal 2001; Eleusenova and Selivanov 1973; Glebova 1992; Glazyrina et al. 2007). However, special analysis of dynamics of plants' interaction with mycorrhizal fungi during vegetation development in man-made habitats is also of considerable importance (Juwarkar and Jambhulkar 2008; Tian et al. 2009). Therefore, the objective of this research article is to investigate the patterns of change in the ratio of different mycorrhizal plants over a period of three decades and accelerated overgrowth of coal mine overdump in the subzone of the dry steppes of Kazakhstan.

2 Materials and Methods

2.1 Site Description

Data obtained during observations on the east dump of the overburden rocks of Fedorovsky open-pit mine (Central Kazakhstan, Karaganda, 49°50′N, 72°51′E) were analyzed (Fig. 1). The climate is continental with hot summer and cold small snowy winters (Agroclimatic facility Karagandy region 1976). The average annual

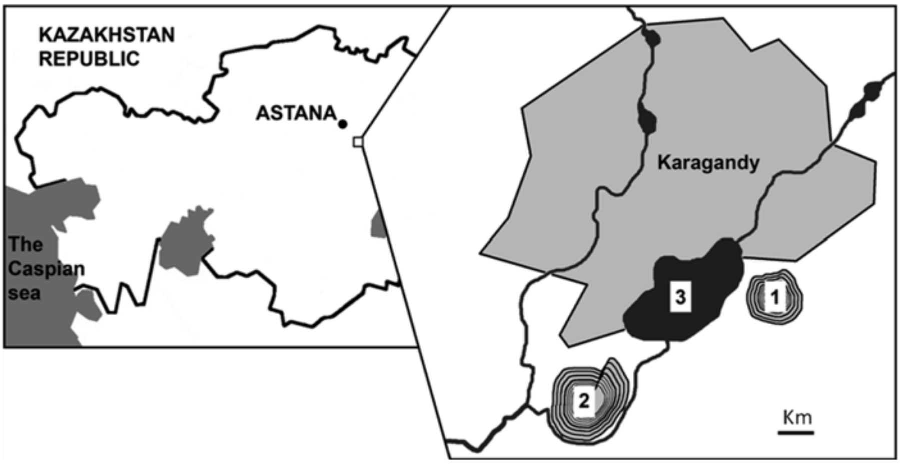

Fig. 1 The map of investigated coal mine (Fedorovsky open-pit mine) overburden of Karaganda, Kazakhstan. *1* East dump; *2* west dump; *3* reservoir in the site of the flooded coal mine

temperature is +3.6 °C. The coldest month is January (average temperature of −13.5 °C), the warmest – July (+20.8 °C). The annual sum of positive temperatures above +10 °C is 2,200–2,250 °C. Average annual rainfall is 315 mm, most of which falls between May and July. Low-powered snow cover, the height of which does not usually exceed 10–20 cm, is held from November to March, an average of 145 days a year. Freezing of soils on the upland reaches 2 m. It is characterized by high variability of the weather – the winter thaws and frosts in the summer.

The bedrocks of the study area are clay, shale, limestone, marl, and other basic rocks, which are characterized by a heavy mechanical structure and usually saline (Storozhenko 1952). The soil cover consists mainly of dark chestnut soils and salt marshes (Storozhenko 1952, 1967; Evstifeev 1959). In the immediate proximity of the dump are common steppe salt licks in the multicolored clays with thin (up to 6 cm) humus-eluvial horizons and dense saline illuvial horizons with a columnar structure. Reserves available to plant moisture content are usually stored in the soils until the third decade of May, and summer moisture only appears after rainfalls (Miroshnichenko and Buevich 1976).

Zonal vegetation is represented by steppe communities (Lavrenko and Borisov 1976; Miroshnichenko and Buevich 1976; Selivanov et al. 1964). Dark chestnut soils are the most common dry steppes dominated by *Stipa capillata* L., *Seseli ledebourii* G. Don, *Artemisia nitrosa* Weber, and *Festuca valesiaca* Gaudin. On low-power soils, communities dominated by *S. capillata*, *F. valesiaca*, *Centaurea sibirica* L., *Onosma simplicissima* L., and *Scorzonera austriaca* Willd. are formed. Saline soils are dominated by halophytic forbs (*Limonium caspium* (Willd.) Gams, *L. gmelinii* (Willd.) Kuntze, *Atriplex cana* C. A. Mey., *Kochia prostrata* (L.) Schrad., and *Camphorosma lessingii* Litv.). Vast areas of waste grounds, roads, and disturbed sites are occupied by weeds, viz., *Kochia scoparia* (L.) Schrad., *Artemisia sieversiana* Willd., *Lactuca tatarica* (L.) C. A. Mey., *Lactuca serriola* L., *Cirsium setosum* (Willd.) Besser, *Atriplex sibirica* L., and *Chenopodium album* L.

The east dump, which has been monitored for natural overgrowth, formed in the years 1941–1978; some parts are not affected by the earthen and other works from 1971. The dump complicated multicolored montmorillonite clays with the inclusion of coal dust, aleurolites, and argillites. The density of rocks is uneven: 1.2–1.3 g/sm^3 in the upper layers and 0.8–0.9 g/sm^3 at a depth of 60–90 sm. The content of the rock dump's slightly hydrolyzed N is 3.5–4.6 mg/100 g soil, mobile forms of P is 0.6–0.9 mg/100 g

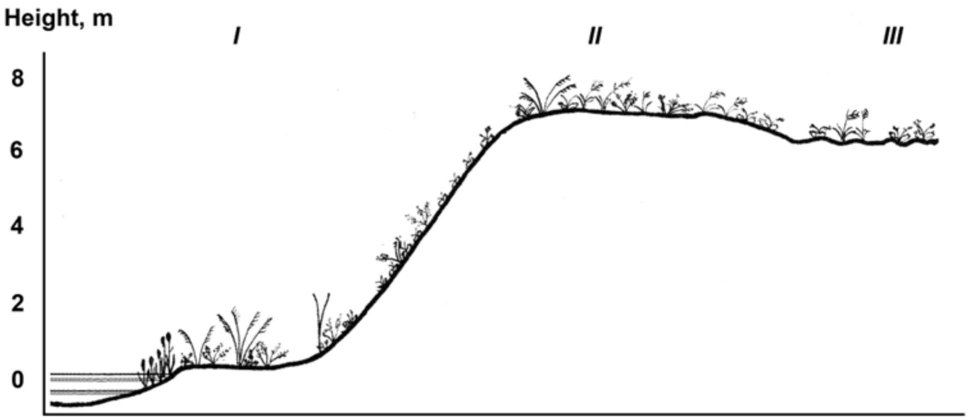

Fig. 2 The location of plots on the dump (*I* near water body, *II* elevated, *III* small uneven)

soil, and mobile forms of K is 8.5–10.0 mg/100 g soil. The average content of major elements of a mineral nutrition (N, P, K) on the heap is 3–10 times lower than in zonal soils. On different plots is observed average and severe sulfatic salinization. The total content of water-soluble sulfates, chlorides, and carbonates ranged from 0.07 % to 1.33 % by weight of absolutely dry soil. The contents of ions varied in the range of 0.6–20.6, 0.3–0.8, and 0.2–0.4 mg-ekv for $HSO4^{2-}$, Cl^{-}, and $HCO3^{-}$, respectively.

Observations on the natural overgrowth of the east dump were carried out in 1978, 1980, 1990, and 2006 (Kuprijanov 1989; Kuprijanov and Manakov 2008) on the same profile length of 1.5 km, covering different areas (Fig. 2). Circumwater plot (I) sometimes submerged by melted water is located at the slope base. Its overgrowth began in 1978 following technogenic alignment. Raised artificially designed plot (II), the overgrowth of which began in 1978, is located on the periphery of the elevated part of the dump. Small uneven planned plot (III), the overgrowth of which began in 1971, is located in the center of the raised part of the dump. On each plot in each period of observation at the sites 10 × 10 m was established the species composition of herbaceous plants. The data for 12 observations (3 plots × 4 time periods) were collected over 36 years. Plots were marked to ensure that the same plots were identified over the whole period of observations. Plot description was carried out in June for each time period to ensure identification of the maximum number of plant species. For each plot, the total number of species regardless of their projective cover was registered. Plant species outside the plot boundaries were ignored.

2.2 Plant Mycorrhizal Status and Plant Nomenclature

Mycorrhizal status of plants is summarized in Table 1. The indirect method for identification of mycorrhizal status was used. The method is based on accumulation of as many data from literature as possible for each plant species (Harley and Harley 1987; Wang and Qiu 2006). Our estimation of mycorrhizal status is mainly based on works carried out in the territory of the former USSR (Kruger 1957; Selivanov et al. 1964; Selivanov and Gavriljuk 1966; Selivanov 1969; Selivanov and Utemova 1969; Eleusenova and Selivanov 1973; Selivanov and Eleusenova 1974; Kruger and Selivanov 1977; Chibrik et al. 1980; Chibrik and Salamatova 1985; Nozadze 1989; Glebova 1992; Mukhin and Betekhtina 2006; Glazyrina et al. 2007). All cited works used the same method for mycorrhiza identification described by Selivanov (1981). The method consists of three steps: (1) maceration of roots in KOH, (2) staining of macerated roots with aniline blue and making of squashed preparations, and (3) counting of fungal structures (arbuscles,

Table 1 Mycorrhizal status of plants on the dump

Plant species	Mycorrhizal status	References
Alismataceae		
Alisma plantago-aquatica L.	noM	Mukhin and Betekhtina (2006)
		Wang and Qiu (2006)
Asteraceae		
Achillea nobilis L.	AM	Indirect identification
Acroptilon repens (L.) DC.	AM	Eleusenova and Selivanov (1973)
Artemisia austriaca Jacq.	AM	Chibrik and Salamatova (1985)
A. dracunculus L.	AM	Glebova (1992)
A. nitrosa Weber	AM	Selivanov and Gavriljuk (1966)
A. proceriformis Krasch.	AM	Indirect identification
A. sieversiana Willd.	AM	Tian et al. (2009)
Chartolepis intermedia Boiss.	AM	Indirect identification
Cirsium setosum (Willd.) Besser	AM	Chibrik and Salamatova (1985)
		Glazyrina et al. (2007)
Erigeron acris L.	AM	Chibrik and Salamatova (1985), Mukhin and Betekhtina (2006), Püschel et al. (2007a, b)
Galatella punctata (Waldst. & Kit.) Nees	AM	Indirect identification
Inula britannica L.	AM	Kruger 1957
Lactuca tatarica (L.) C. A. Mey.	noM-AM	Chibrik and Salamatova (1985), Glebova 1992, Glazyrina et al. (2007)
Saussurea amara (L.) DC.	noM-AM	Selivanov and Gavriljuk (1966), Chibrik et al. (1980)
S. salsa (Pall. ex M. Bieb.) Spreng.	Unknown	
Senecio erucifolius L.	AM	Harley and Harley (1987)
Senecio vulgaris L.	noM-AM	Wang and Qiu (2006)
Sonchus arvensis L.	AM	Chibrik and Salamatova (1985)
Taraxacum officinale F. H. Wigg.	AM	Chibrik and Salamatova (1985), Glebova (1992), Mukhin and Betekhtina (2006), Glazyrina et al. (2007)
Tragopogon capitatus S. A. Nikitin	AM	Indirect identification
Tripleurospermum perforatum (Merat) M. Lainz	AM	Chibrik and Salamatova (1985), Mukhin and Betekhtina (2006)
Tripolium vulgare Nees	AM	Sonjak et al. (2009)
Xanthium strumarium L.	AM	Shah et al. (2009)
Boraginaceae		
Lappula squarrosa (Retz.) Dumort.	noM-AM	Chibrik and Salamatova (1985), Glebova (1992), Mukhin and Betekhtina (2006), Glazyrina et al. (2007)
Brassicaceae		
Brassica campestris L.	noM	Harley and Harley (1987), Wang and Qiu (2006)
Capsella bursa-pastoris (L.) Medikus	noM-AM	Chibrik and Salamatova (1985), Harley and Harley (1987), Mukhin and Betekhtina (2006), Wang and Qiu (2006), Glazyrina et al. (2007)

(continued)

Table 1 (continued)

Plant species	Mycorrhizal status	References
Descurainia sophia (L.) Webb ex Prantl	noM	Eleusenova and Selivanov (1973), Harley and Harley (1987)
Lepidium latifolium L.	noM	Eleusenova and Selivanov (1973), Selivanov and Eleusenova (1974), Harley and Harley (1987)
L. perfoliatum L.	noM	Eleusenova and Selivanov (1973)
L. ruderale L.	noM-AM	Harley and Harley (1987), Glazyrina et al. (2007)
L. songaricum Schrenk	Unknown	
Rorippa palustris (L.) Besser	noM	Wang and Qiu (2006)
Caryophyllaceae		
Gypsophila altissima L.	noM	Selivanov et al. (1964)
G. perfoliata L.	Unknown	
Silene suffrutescens M. Bieb.	noM	Eleusenova and Selivanov (1973)
Chenopodiaceae		
Atriplex littoralis L.	AM	Wang and Qiu (2006)
A. sagittata Borkh.	noM	Püschel et al. (2007a, b)
A. sibirica L.	Unknown	
Bassia sedoides (Pall.) Asch.	Unknown	
Halimione verrucifera (M. Bieb.) Aellen	noM-AM	Selivanov et al. (1964), Selivanov and Gavriljuk (1966)
Kochia scoparia (L.) Schrad.	noM	Pendleton and Smith (1983)
Petrosimonia sibirica (Pall.) Bunge	Unknown	
P. triandra (Pall.) Simonk.	Unknown	
Salsola collina Pall.	noM	Wang and Qiu (2006)
S. foliosa (L.) Schrad.	noM	Indirect identification
S. nitraria Pall.	noM	Indirect identification
S. tamariscina Pall.	noM	Eleusenova and Selivanov (1973)
Suaeda altissima (L.) Pall.	noM	Eleusenova and Selivanov (1973)
Convolvulaceae		
Convolvulus arvensis L.	noM-AM	Chibrik and Salamatova (1985), Harley and Harley (1987), Glebova (1992), Mukhin and Betekhtina (2006), Wang and Qiu (2006)
Cyperaceae		
Bolboschoenus maritimus (L.) Palla	noM-AM	Eleusenova and Selivanov (1973), Druva-Lusite and Ievinsh (2010)
Carex obtusata Lilj.	noM	Selivanov (1969), Selivanov and Utemova (1969)
C. supina Willd. ex Wahlenb.	AM	Nozadze (1989)
Scirpus sylvaticus L.	noM	Nozadze (1989)
Fabaceae		
Astragalus testiculatus Pall.	AM	Eleusenova and Selivanov (1973)
Glycyrrhiza uralensis Fisch.	AM	Liu et al. (2007)
Lotus krylovii Schischk. & Serg.	AM	Indirect identification
Medicago falcata L.	AM	Wang and Qiu (2006)
Melilotus albus Medikus	noM-AM	Chibrik and Salamatova (1985), Glazyrina et al. (2007)

(continued)

Table 1 (continued)

Plant species	Mycorrhizal status	References
M. officinalis (L.) Pall.	AM	Kruger and Selivanov (1977), Wang and Qiu (2006)
Geraniaceae		
Geranium collinum Stephan ex Willd.	AM	Indirect identification
Juncaceae		
Juncus gerardii Loisel.	noM-AM	Selivanov et al. (1964), Wang and Qiu (2006)
J. nastanthus V. I. Krecz. & Gontsch.	Unknown	
Lamiaceae		
Phlomoides tuberosa (L.) Moench	AM	Chibrik and Salamatova (1985)
Lythraceae		
Lythrum virgatum L.	Unknown	
Onagraceae		
Epilobium palustre L.	AM	Harley and Harley (1987)
Plantaginaceae		
Plantago major L.	noM-AM	Chibrik and Salamatova (1985), Mukhin and Betekhtina (2006), Glazyrina et al. (2007)
Plantago media L.	AM	Kruger (1957), Wang and Qiu (2006)
Plumbaginaceae		
Limonium caspium (Willd.) Gams	noM	Selivanov and Gavriljuk (1966)
L. gmelinii (Willd.) Kuntze	noM	Selivanov and Gavriljuk (1966)
Poaceae		
Agropyron cristatum (L.) P. Beauv.	AM	Chibrik and Salamatova (1985)
Agrostis gigantea Roth	AM	Chibrik and Salamatova (1985)
Calamagrostis epigeios (L.) Roth	AM	Chibrik and Salamatova (1985), Glazyrina et al. (2007)
Elytrigia geniculata (Trin.) Nevski	AM	Indirect identification
E. repens (L.) Nevski	AM	Chibrik and Salamatova (1985), Glebova (1992), Mukhin and Betekhtina (2006)
Eremopyrum triticeum (Gaertn.) Nevski	AM	Shi et al. (2006)
Hordeum brevisubulatum (Trin.) Link	AM	Selivanov et al. (1964)
H. jubatum L.	AM	Wang and Qiu (2006)
Leymus paboanus (Claus) Pilg.	AM	Selivanov et al. (1964)
L. racemosus (Lam.) Tzvelev	AM	Eleusenova and Selivanov (1973)
Phragmites australis (Cav.) Trin. ex Steud.	noM-AM	Chibrik and Salamatova (1985)
Poa angustifolia L.	AM	Kruger (1957), Wang and Qiu (2006)
Puccinellia distans (Jacq.) Parl.	noM-AM	Selivanov et al. (1964), Wang and Qiu (2006)
P. tenuiflora (Griseb.) Scribn. & Merr.	AM	Indirect identification
P. tenuissima Litv. ex V. I. Krecz.	AM	Selivanov et al. (1964)
Stipa lessingiana Trin. & Rupr.	AM	Nozadze (1989)
Typha angustifolia L.	noM-AM	Wang and Qiu (2006)

(continued)

Table 1 (continued)

Plant species	Mycorrhizal status	References
Polygonaceae		
Polygonum aviculare L.	noM-AM	Selivanov and Eleusenova (1974), Chibrik and Salamatova (1985), Mukhin and Betekhtina (2006), Glazyrina et al. (2007)
P. bordzilowskii Klokov	Unknown	
Rumex halacsyi Rech.	Unknown	
R. stenophyllus Ledeb.	Unknown	
Ranunculaceae		
Ranunculus sceleratus L.	noM-AM	Wang and Qiu (2006)
Rosaceae		
Potentilla supina L.	AM	Indirect identification
Salicaceae		
Populus balsamifera L.	AM-ECM	Wang and Qiu (2006)
Salix caspica Pall.	AM-ECM	Indirect identification
Solanaceae		
Hyoscyamus niger L.	AM	Glazyrina et al. (2007)
Tamaricaceae		
Tamarix ramosissima Ledeb.	AM	Eleusenova and Selivanov (1973)

AM arbuscular mycorrhizas, *AM-ECM*, arbuscular and ectomycorrhizas
Unknown – unable to install mycorrhizal status, noM – non-mycorrhizal species, noM-AM – variable or optional mycorrhizal species

vesicles, and hyphae) under a microscope. Most of the published papers mentioned only the presence or absence of mycorrhiza without details of fungal structures. For the identification of mycorrhizal status of one to two plant species, data from papers outside the former USSR (Pendleton and Smith 1983; Shi et al. 2006; Liu et al. 2007; Püschel et al. 2007a, b; Shah et al. 2009; Sonjak et al. 2009; Tian et al. 2009; Druva-Lusite and Ievinsh 2010) were used.

For the final characteristic of mycorrhizal status of plants, all available information was used. Data from all sources were treated as reliable. No published data could be found for 13 plant species. In this case, plant species were considered to be mycorrhizal if other closely related species were able to form mycorrhiza.

Species for which all publications mentioned the lack of mycorrhizas are believed to be nonmycorrhizal (noM). The species was considered as facultatively mycorrhizal (noM-AM) when they was in some sources appear as mycorrhizal, while others – like not form mycorrhizas. Species that form arbuscular mycorrhizas in compliance with all the sources are believed to be obligately mycorrhizal (Frost et al. 2001). Species both with arbuscular mycorrhizas and with ectomycorrhizas were considered as mycorrhizal species at the analysis stage. Nomenclature and authorities are used as by Cherepanov (1995).

2.3 Data Analysis

To assess the significance of dynamics of the representation of plant species of different mycorrhizal status in the course of succession, the nonparametric Spearman correlation coefficient (r_{SP}) was used. For an analytical description of the dynamics of representation of plants species of the different mycorrhizal status in the course of succession, the logistic curve was used given by the expression

$$P = \frac{A - a0}{1 + \exp(\alpha + \beta x)} + a0,$$

where P is a share of species group, x is the time from the start of self-organized vegetation (years), α and β are coefficients, and A and $a0$ are empirically set minimum ($a0$) and maximum (A) levels of function. Coefficients were found by a method of iterative numerical estimation. Coordinates of critical points of infection of functions (x_{lower} and x_{upper} points), marking the end of the rapid changes of function values, were found in accordance with recommendations by Vorobeychik et al. (1994).

3 Results

By the time of the last registration in 2006, plots I and II were overgrown for 29 years, and plot III for 36 years. During this time, at the dump there were formed the communities are less diverse in the floristic terms compared with the zonal plant communities. The total projective cover of plants in the last accounting period in 2006 was 30–40 %. High participation of Brassicaceae and Chenopodiaceae and less abundance of gramineous, forbs and ephemeroids is characteristic feature of dump's plant communities (Kuprijanov and Manakov 2008). The presence of halophilic species such as *Bassia*, *Halimione*, *Limonium*, *Petrosimonia*, *Salsola*, *Saussurea*, and *Suaeda* reflects expressiveness of salinization processes, particularly evident on plot III.

From the 97 registered species on three plots for 85 species mycorrhizal status was set. Species for which on the literary data failed to find the characteristics of the relationship with mycorrhizal fungi most often refer to the Polygonaceae (three out of four recorded species of the family) and Chenopodiaceae (4 of 13 registered species of the family). A total of non-mycorrhizal are 19 species of plants, 17 species are facultatively mycorrhizal, 47 form arbuscular mycorrhizas, and 2 woody plants species form both arbuscular endomycorrhizas and ectomycorrhizas. Thus, 78 % of species for which the type of interact with mycorrhizal fungi is set have mycorrhizal colonization. This value (78 %) is less than estimates of the share of mycorrhizal species in the subzone of northern forest steppe (90 %; Selivanov et al. 1964), but is higher than the known estimates for the northern deserts of Kazakhstan (65 %; Eleusenova and Selivanov 1973). The greatest number of non-mycorrhizal species, among indigenous for heap, belongs to the Brassicaceae and Chenopodiaceae and mycorrhizal ones mainly include representatives of the Asteraceae and Poaceae (Table 2). Such distribution is to be expected, since this is observed often (Selivanov 1981; Wang and Qiu 2006).

The dynamics of plant species of different mycorrhizal status during self-organized vegetation of dump is described in Table 3. At plots I and II the first descriptions were made in the beginning year of overgrowing (1978). Therefore a increase of the total number of recorded species at these plots was well expressed (from 4 to 21 species in 1978 to 28–33 species in 2006). Dumping of the plot III was done in 1971, that is why observations of the first dynamic stages of overgrowth were not carried out, therefore at this plot a increase of the species number was not expressed (Table 3).

On plots I and II during the first 3 years of observations in varying degrees, the number of species of all categories of mycotrophy increased. And over the next 26 years, only the number of mycorrhizal species increased, while the number of non-mycorrhizal and changeable mycorrhizal species during this time reduced or remained unchanged. For plot III during the observation period, an almost double decrease in the number of species of all groups of mycotrophy and, accordingly, the total number of plant species was registered – from 67 species in 1978 to 36 in 2006 (Table 3).

Grass ecosystems are characterized by considerable fluctuation in species composition (Mirkin and Naumova 1998). During the observation period, properties of ecotopes influencing the settlement and survival of plants changed (consolidation of substrates and increase of salinization were registered) (Kuprijanov and Manakov 2008). Therefore it is more justified to analyze not absolute number of different plant groups but their share. In preparation for such comparison we have used different durations of the vegetation development on plots I–II on one hand and on plot III on another (Fig. 3).

Table 2 Representation of species of different mycorrhizal status in large families

Family	Mycorrhizal status Non-mycorrhizal (noM)	Facultative mycorrhizal (noM-AM)	Mycorrhizal (AM + AM-ECM)
All species	19 (100)	17 (100)	49 (100)
Including:			
Asteraceae	0 (0)	3 (18)	19 (39)
Brassicaceae	5 (26)	2 (12)	0 (0)
Chenopodiaceae	7 (37)	1 (6)	1 (2)
Cyperaceae	2 (11)	1 (6)	1 (2)
Fabaceae	0 (0)	1 (6)	5 (10)
Poaceae	0 (0)	3 (18)	14 (29)

Percentage (%) of the total number of species of present status is given in parentheses
AM arbuscular mycorrhizas, *AM-ECM* arbuscular and ectomycorrhizas
noM – non-mycorrhizal species, noM-AM – variable or optional mycorrhizal species

Table 3 Changing the number of species of different mycorrhizal status during self-organized vegetation of the dump

Mycorrhizal status	Year 1978	1980	1990	2006	Total for 1978–2006
Plot I					
Unknown status	5	6	5	4	6
Non-mycorrhizal (noM)	2	3	2	2	5
Facultative mycorrhizal (noM-AM)	8	10	5	5	10
Mycorrhizal (AM + AM-ECM)	6	16	16	17	20
All species	21	35	28	28	41
Plot II					
Unknown status	0	4	3	3	5
Non-mycorrhizal (noM)	2	8	2	4	12
Facultative mycorrhizal (noM-AM)	1	7	3	5	9
Mycorrhizal (AM + AM-ECM)	1	11	10	21	24
All species	4	30	18	33	50
Plot III					
Unknown status	7	7	5	4	8
Non-mycorrhizal (noM)	13	13	9	7	13
Facultative mycorrhizal (noM-AM)	12	12	9	6	12
Mycorrhizal (AM + AM-ECM)	35	35	25	19	38
All species	67	67	48	36	71

AM arbuscular mycorrhizas, *AM-ECM* arbuscular and ectomycorrhizas
Unknown – unable to install mycorrhizal status, noM – non-mycorrhizal species, noM-AM – variable or optional mycorrhizal species

At plots I and II, the share of mycorrhizal species on a measure of self-organized vegetation increased approximately twice from 25–38 % in 1978 to 61–70 % in 1990–2006. The difference between these plots consists in the following aspects: On plot I in the process of vegetation development, the share of optional mycorrhizal species mainly decreased (from 50 % in 1978 to 21–22 % in 1990–2006). On plot III, the observation showed that the plants' proportions of different mycorrhizal status for the 30-year-old period of observation did not change despite essential change of the general floristic structure of plants (Kuprijanov and Manakov 2008).

The similarity of the highlights of the temporal dynamics of participation of different mycotrophy on different parts of dumping allows to combine data and analyze them together. A pro-

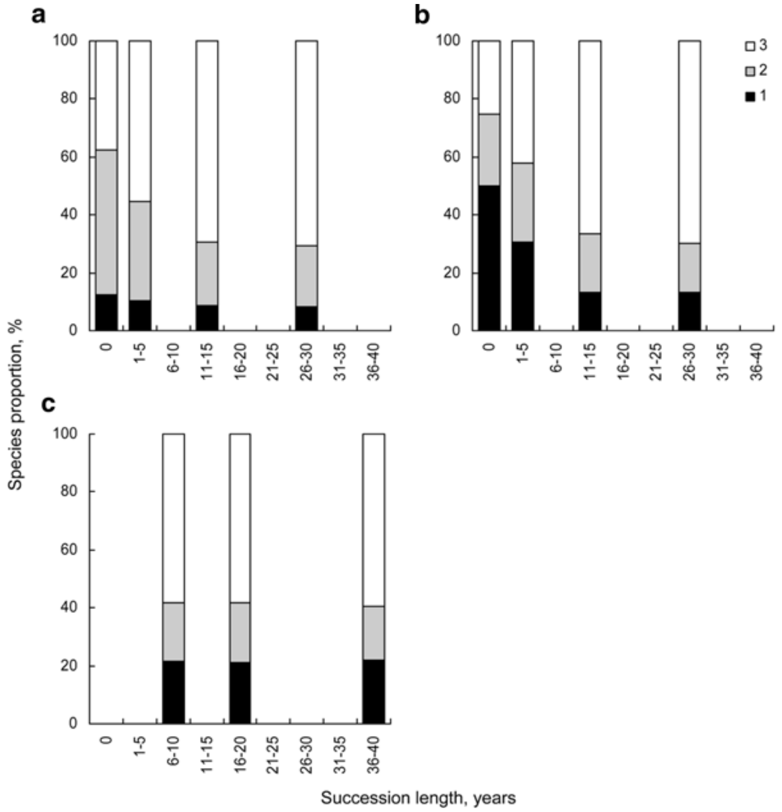

Fig. 3 **a**–**c** Changes in self-organized vegetation on plots I (**a**), II (**b**), and III (**c**) of the dump in proportion to species of different mycotrophic status (*1* non-mycorrhizal, *2* facultative mycorrhizal, *3* mycorrhizal)

portion of non-mycorrhizal plant species over time is reduced but to the value of the Spearman correlation coefficient is insignificant ($r_{\mathrm{SP}} = -0.23$, $P = 0.4657$). Satisfactory approximation of dependence of the logistic curve (Fig. 4a; $R^2 = 0.61$; $P = 0.0034$) indicates the nonlinear nature of the process. Stabilization of the share of non-mycorrhizal species occurs at 7–8 years of overgrowth (abscissa of lower critical point $x_{\mathrm{lower}} = 7.5$). A proportion of optional mycorrhizal species along the succession is reduced statistically significant ($r_{\mathrm{SP}} = -0.76$, $P = 0.0039$) and is expressed nonlinearly (Fig. 4b; for the logistic curve $R^2 = 0.61$; $P < 0.0001$). In this case the stabilization of the share of non-mycorrhizal species occurs at 4–5 years of overgrowth ($x_{\mathrm{lower}} = 4.2$). The proportion of obligate mycorrhizal species increases during succession statistically significant ($r_{\mathrm{SP}} = +0.84$, $P = 0.0007$) and is expressed nonlinearly (Fig. 4b; for the logistic curve $R^2 = 0.80$; $P < 0.0001$). In this case, the stabilization of the share of optional non-mycorrhizal species occurs at 3–4 years of overgrowth ($\mathrm{x}_{\mathrm{upper}} = 3.5$).

4 Discussion

The obtained data indicate that the increase in proportion of mycorrhizal plant species observed in natural endoecogenetic successions (Miller 1979; Gemma and Koske 1990; Ahulu et al. 2005; Püschel et al. 2007a, b), is well reproduced and statistically confirmed in the course of succession in unfavorable man-made habitats. Our observations of the development communities for

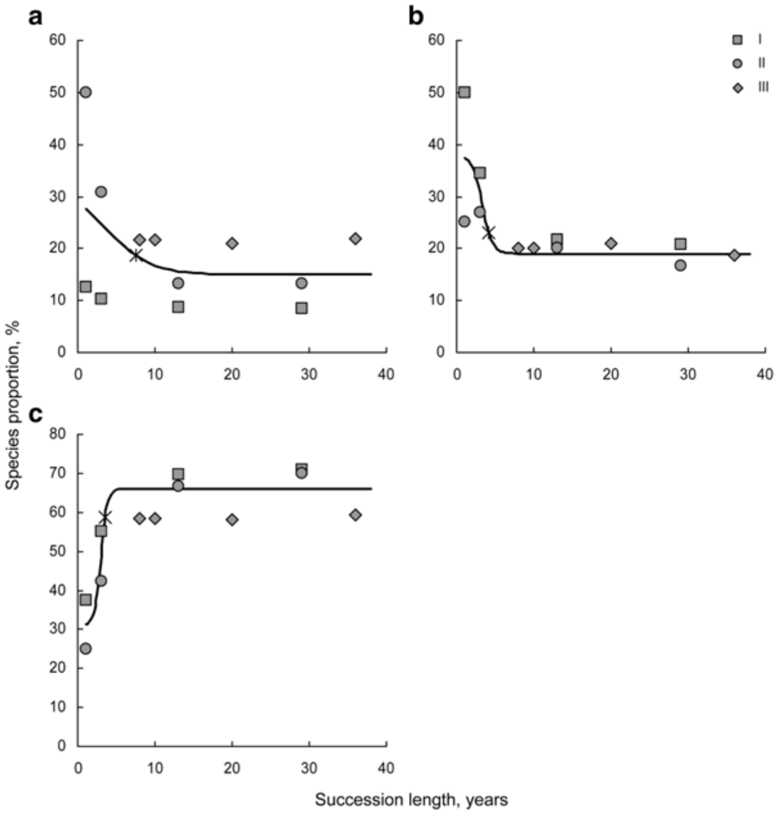

Fig. 4 **a**–**c** Approximation of the relationship between fractions of non-mycorrhizal (**a**), facultative mycorrhizal (**b**), and mycorrhizal (**c**) plant species and duration of succession in different parts of the dump (I, II, and III – plots) by logistic curves. *Asterisk* – position of the *bottom* (**a**, **b**) and *top* (**c**) of critical points marking the end of a period of rapid change of indicators

which absolute (in years) duration of existence is known have allowed to characterize quantitatively the rate of change in the ratio of plants of different groups of mycotrophy. We believe precise dating of events occurring during the formation of communities to be a significant advantage for the observation of successions on artificial anthropogenic substrates. According to our data, the ratio of the number of species of different groups of mycotrophy stabilizes fairly quickly. Within the first 5 years of overgrowth, the share of mycorrhizal species reaches about 60–70 % on all plots and doesn't subsequently change.

The observed changes are explained with the help of two hypotheses. The first assumption is that there is lower competitiveness of non-mycorrhizal species as compared with mycorrhizal ones. Greater competitiveness of mycorrhizal species was demonstrated experimentally (Heijden et al. 1998, 2003), and this explanation is accepted in part of studies, describing successions in natural communities (Pezzani et al. 2006; Püschel et al. 2007a). The second assumption is that the ratio of different types of mycotrophy in the course of succession may depend only on their rate of settlement, i.e., ruderal, or on the level of reactivity of different species. The positive relationship between ruderal of Grime et al.'s (1988) environmental strategy and the avoidance of mycorrhizal fungi has been discussed previously (Grime et al. 1988; Francis and Read 1995; Cornelissen et al. 2001). There is a known pattern of increased occurrence of non-mycorrhizal species among species with ruderal strategy as compared with competitive and mixed environmental strategies (Grime et al. 1988; Betekhtina and Veselkin 2011). Our data do not allow arguments to accept or reject any of the two hypotheses, but the second hypothesis has a number of circumstantial evidence.

Extremeness of conditions of the investigated dump is caused by several factors (Kuprijanov and Manakov 2008): firstly, high density of clay substrates prevents seed reproduction on many species; secondly, moderate and severe salinization and low content of elements of mineral nutrition; and thirdly, lack of water availability on plots II and III. In conformity with the polymodelling concept of phytocenoses organization (Mirkin and Naumova 1998) studied communities best meet the abiotic S-model describing communities of plants-patients (stress tolerants by Grime et al. 1988) in extreme abiotic conditions. In such circumstances, competition is weakened, and the structure and dynamics of vegetation communities is determined in first place by regularities of populational dynamics of individual species. To describe the successions in these communities, the most suitable is the model of neutrality (Mirkin and Naumova 1998). The applicability of this model to the studied communities is confirmed, in particular, by observations of *Glycyrrhiza uralensis*, *Medicago falcata*, *Silene suffrutescens*, *Stipa lessingiana*, and some other plants typical of zonal communities. On plot III, these species have settled in the first favorable settlement for the period before start of observations, i.e., in the interval between 1971 and 1978. However, over further 30 years, their seed reproduction was not registered, probably due to increasing unfavorable abiotic conditions. Therefore, these species felt out of communities. Also, the pessimal conditions (mostly salinization) are probably a reason that to the dump such conventional weed-steppe species with traits of competitive strategy as *Centaurea cyanus* L. and *Echinochloa crus-galli* (L.) Beauv. and typical steppe plants such as *Festuca valesiaca* were not found (Kuprijanov and Manakov 2008).

In compliance with the model of a neutral flow of successions it can suggest the following explanation of the observable facts. From the local pool of species most of them (probably 70–85 %) are mycorrhizal (Selivanov et al. 1964; Eleusenova and Selivanov 1973); many species consistently colonize dump. But the penetration rate in the new location of non-mycorrhizal pioneer ruderals (e.g., species Brassicaceae and Chenopodiaceae), ruderals with features of stress tolerants (halophilic Chenopodiaceae) are higher than the rate of penetration of mycorrhizal perennials from Asteraceae, Fabaceae, and Poaceae families. This explains the predominance of non-mycorrhizal and facultative mycorrhizal plant species in the first period of colonization. Subsequent growth of the mycorrhizal species is explained by the occupancy of new mycorrhizal species. Disappearance of non-mycorrhizal species from species counts is expressed not clearly (see Table 3). Over time, the composition of species that can exist in harsh abiotic conditions of the dump stabilized, and the prevalence in it of mycorrhizal plants reflects their dominance in the local flora as a whole. It seems that competitive advantages of mycorrhizal plants may be more important in determining the abundance of species at plots, i. e., of their participation in the creation of a common covering or biomass of communities.

In conclusion, we consider it necessary to draw attention to the important fact for correct extrapolation of described conformity to natural laws on other situations. The subject of analysis in this report is mycorrhizal status of plant species. This characteristic, which is separated from the concrete conditions, describes only the probability of the introduction of the individual or individuals of cenopopulation in interaction with mycorrhizal fungi. There are cases where mycorrhizas were found sporadically in plants considered universally non-mycorrhizal everywhere and conversely were not found at the usual mycorrhizal plants. The last is probably especially true for abiotic adverse habitats (Nozadze 1989) including technogenic (Chibrik et al. 1980) or disturbed pioneer habitats (Miller 1979). Thus, our data cannot be directly interpreted as an indication of changing in the course of succession tightness of interaction of plants with arbuscular fungi in the investigated dump. They only show that during spontaneous development of vegetation, the ratio of plants that are able to cooperate with mycorrhizal fungi naturally and steadily changes. These changes lead to dominance for a long time of developing phytocenoses of the investigated dump, an organization of which is well described by abiotic S-model of obligate mycorrhizal species of herbaceous plants.

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Part IV

Green Economy and Green Nanotechnology

Drivers of Green Economy: An Indian Perspective

Sanjay Kumar Kar, Saroj Kumar Mishra,
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Abstract

Sustainable economic development is much needed to ensure human well-being and reduce social inequality. In recent times, global leaders have come closer to arrive at consensus to transform current unsustainable economic developments into sustainable green economic growth. Viability of green economy depends on several factors such as government policy, investment climate, and concerns for environment. This chapter discusses drivers of green economy and the role of renewables to drive green growth in India.

Keywords

Fossil fuels • Green fuels • Natural gas • Renewable energy

1 Green Economy

Green economy is defined by the United Nations Environment Programme (UNEP) as one that "focuses on improving human well-being and reducing social inequity over the long-term, while not exposing future generations to significant environmental risks and ecological scarcities" (UNEP 2011). The current global ecosystem seems to be unsustainable and facing financial, economic and environmental crises (Cai et al. 2011) and to remedy lingering stagnation from the financial crisis, key actors are proposing revolutionary economic reforms (Lane 2010). The recent global crises sensitised people to move away from brown economy to green economy. Mounting evidence also suggests that transitioning to a green economy has sound economic and social justification (UNEP 2011).To a large extent, global political leaders are showing concerns over unsustainable economic growth, and many of them are in favour of a new sustainable economy based on much desirable green growth (Hamdouch and Depret 2010). Green growth is quality-oriented, low-carbon, energy-efficient growth with a focus on creating value through clean technology, natural infrastructure and innovation in markets for environmental goods and services (Vazquez-Brust and Sarkis 2012). Some of the leading developed countries and developing countries are making serious

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progress towards green economy. For example, the largest developing country in the world, China is also acting positively to catch up with the green economy wave. From 2006 to 2009, China had cumulatively closed down 60.4 gigawatts (GW) of small power plants and 82 million tons (MT), 60 MT and 214 MT of inefficient iron, steel and cement production facilities, respectively (Cai et al. 2011). Renewables consumption in China had increased from 0.8 million tons of oil equivalent (Mtoe) in 2002 to 25.4 Mtoe in 2012. In China, rapid development of renewable energy has made great contributions to emission reduction and job growth (Cai et al. 2011). The authors estimated that with every per cent increase in the share of solar PV generation there will be a 0.68 % increase of total employment in China. Similarly, every 1 % increase in share of biomass and wind will have 0.2 % and 0.05 % incremental impact on total employment, respectively. It is desirable that government and administrations at various levels should devise policies and efficiently implement them to promote the idea of green economy. de Oliveira et al. (2013) suggest that reform and policy changes in the city level are critical as cities are drivers of the global economy because they are the centre of knowledge and innovations (both technological and institutional) essential for viability of green economy. Good governance is another essential component of proper functioning of green economy. In order to develop and protect green economy, administrations should keep track of sustainable production, resource use efficiency, responsible and sustainable consumption, internalisation of externalities and resource conservation. Because of nonrenewable resource-driven policies, many countries have incentivised corporates, individuals and society at large to deplete natural resources without hesitation. Sustainable production and consumption has been viewed as a loss-making proposition by many stakeholders including the government. Now, time has reached to reverse the trend and promote sustainability. Governments, regulators and administrators should create a level playing field for greener products by "phasing out antiquated subsidies, reforming policies and providing new incentives, strengthening market infrastructure and market-based mechanisms, redirecting public investment, and greening public procurement" (UNEP 2011). A quick transition to a green economy is not only necessary but a must. This can assist in overcoming the contribution that population growth makes to the depletion of scarce natural resources. The world's least developed countries (LDCs) are significantly affected by environmental degradation than most other developing countries (UNCTAD 2010), so early transition to a green economy could prove beneficial to them. In June 2012, world leaders envisioned at the Rio + 20 conference to strive for sustainable development. They reaffirmed "international trade [as] an engine for development and sustained economic growth", and identified the green economy as "an important tool for achieving sustainable development" (UNEP 2013). Developing countries like India should proactively and purposefully strive for a green economy driven by renewable energy.

2 Renewable Energy Environment in India

2.1 Political/Legal Environment

The political and legal environment could significantly impact the renewable energy market development, especially in a country like India where pricing of competing fuel is still to some extent being controlled by the government. Any decision related to pricing is not free from political intervention. Such decisions may have serious implications for promotion of renewable energy. For example, complete deregulation of diesel may have greater impact on the opportunities and threats for the renewable market in the country. After partial deregulation of diesel price, electricity generated from renewables could compete with electricity produced from diesel-based generators. Similarly, revision of domestic gas price is due in 2014 and could impact the growth of renewable energy production and consumption. The domestic gas price is most likely to be revised from the current level of $4.2/MMBtu to about $8/MMBtu. This would increase competitiveness of renewable energy. Political decisions related to subsidised fossil fuel negatively impact renewable market development, and subsidy on renewable energy may prove a driver for renewable market. Government regulations on mandatory purchase of renewable energy to feed the grid network promote production and consumption of renewable energy in the country.

2.2 Economic Environment

Higher disposable income combined with higher affordability and accessibility to energy-consuming products like air conditioners, refrigerators, and heaters increases energy consumption. The economic environment in many ways impacts different industries including the energy industry. For example, petroleum and natural gas industry in India has been traditionally and historically exposed to various economic challenges like high growth rate, rate of interest, inflation and fluctuation in currency exchange rates. Overdependency on import of petroleum products means higher exposure to currency fluctuations leading to price fluctuations in the domestic market. Liquified natural gas (LNG) import from spot markets seriously impacts the final price of natural gas in India, hence influencing the consumption pattern. Though in the recent past there have been successful finds of natural gas reserves like the KG basin in India, still a significant demand is met through imported LNG. Thus, the international LNG prices, shipping costs, geopolitical ties between the countries and other international events have a direct or indirect impact on energy pricing in India. The recent weakening of the Indian currency versus the US dollar seriously dented the current account deficit of the country. The government has been encouraging consumers to consume less fossil fuel-based energy and use more renewable forms of energy.

2.3 Social/Demographic Environment

In recent times, the consumer preference over energy choice has been changing. Well-informed consumers are getting attracted towards greener fuel like renewables and natural gas due to less pollution and due to it being an environmentally safer option. Industrial and commercial customers are looking for a reliable and efficient supply of energy along with other environment benefits (Kar and Sahu 2012). On the other hand, the transport sector has been searching for economical, safer and environment-friendly fuel like compressed natural gas (CNG). It is clear that the social/demographic environment is steadily pushing for the adoption of greener technologies. Consumers across the globe are becoming far more concerned about energy efficiency and intensity, water footprint and carbon footprint which have direct linkage with climate changes. Even Indian consumers are gradually looking for green options, be it industrial and domestic customers in favour of natural gas and individual buyers looking for energy-saving white goods.

2.4 Technological Environment

Technological upgradations have been very much visible in various forms of energy technology. Advanced technology in the energy domain has been used to enhance production, efficiency and utilisation of available resources. Technology has been continuously evolving across the energy value chain. For example, the steady technology upgradation is clearly visible in petroleum and natural gas value chain starting from exploration and production to marketing of finished products. Similarly, there has been constant technological upgradation in the renewable energy sector. In the case of wind technology, the emergence of rotors designed for lower wind speeds, having even smaller specific power, with high masts and long blades in relation to generator size and even higher capacity factors (IEA 2013), has been the outcome of technology upgradation. This development enables installing wind turbines in lower-wind-speed areas, which are often closer to consumption centres than the best “windy spots”. Due to this installation in environment sensitive places and landscape integration (seashores, mountain ridges, etc.) areas can be avoided leading to lower potential for opposition and conflicts (Chabot 2013). More green technological progress, which may come about in the future, is likely to reduce labour-saving technical change if these compete for the same research resources (Droste-Franke et al. 2012). According Crown Estate (2012), there is potential reduction of 39 % in cost of offshore wind energy in Europe and a significant cost reduction (17 %) will be through new and improved turbines (IEA 2013).

3 Primary Energy Consumption

According to the BP Statistical Review of World Energy (2013) in 2012, China consumed 21.92 % (Table 1) of global primary energy followed by the USA (17.7 %), the Russian Federation (5.5 %) and India (4.52 %). Asia-Pacific with 40 % share of primary energy was the leading region followed by Europe and Eurasia (23.47 %) and the Middle East (6.11 %). In the Asia-Pacific region, coal (52.26 %) was the largest form of primary energy followed by oil (27.83 %) and natural gas (11.26 %). In the region renewables and hydro contributed just about 0.7 %. Unlike Asia-Pacific, Europe and Eurasia is driven by natural gas (33.29 %) closely followed by oil (30 %), and North America is primarily driven by oil (37.30 %) closely followed by natural gas (30 %) (Table 1). In 2012, India with 20.5 billion cubic metre (BCM) became the fourth largest importer of liquefied natural gas (LNG) after Japan (118.8 BCM), South Korea (49.7 BCM) and Spain (21.4 BCM), and India accounts for 6.2 % of the total LNG trade (BP Statistical Review 2013).

4 Renewable Energy Status

Renewable energy is growing steadily and is most likely to become the backbone of a secure and sustainable energy supply in an increasing number of developed and developing countries. Non-fossil fuels are growing quickly but from a very small base, with optimistic estimates placing them at 30 % of the energy mix by mid-century (Kalicki and Goldwyn 2013), and a significant contribution would be from renewables. Many countries have been revisiting their energy policy keeping climate change in mind, and renewables are now given much needed impetus. Many countries are setting ambitious targets in terms of increasing share of renewable energy in the energy mix. Sweden and Norway have set a target to achieve 50 % and 67.5 % of total final energy from renewables by 2020, respectively (Table 2). The Chinese government has ambitious targets for wind, solar and hydro and plans to increase the share of non-fossil fuels to 30 % of installed electricity generating capacity by the end of 2015 (Shuo 2013).

It is observed that with fairly stable and tested technology renewables such as wind and solar are increasingly getting adopted across various markets especially for producing electricity. In 2012, renewable forms of energy accounted for 2.4 % of global energy consumption, up from 0.8 % in 2002; renewables in power generation accounted for a record 4.7 % of global power generation (BP Statistical Review 2013). According to a recent report by REN 21 total renewable power capacity worldwide exceeded 1,470 GW in 2012, up about 8.5 % from 2011. During the 5-year period of 2008–2012, installed capacity of many renewable energy technologies grew very rapidly, with the fastest growth in the power sector (REN21 2013). Total capacity of solar photovoltaic system (PV) grew at rates averaging 60 % annually. Concentrating solar thermal power (CSP) capacity grew more than 40 % per year on average, growing from a small base, and wind power increased 25 % annually

Table 1 Global primary energy consumption (million tonnes oil equivalent) by fuel in 2012

	Oil	Natural gas	Coal	Nuclear energy	Hydro electric	Renewables	Total	Share (%)
USA	819.9	654.0	437.8	183.2	63.2	50.7	*2,208.8*	17.70
Canada	104.3	90.6	21.9	21.7	86.0	4.3	*328.8*	2.64
Mexico	92.6	75.3	8.8	2.0	7.1	2.0	*187.7*	1.50
Total North America	*1,016.8*	*820.0*	*468.5*	*206.9*	*156.3*	*57.0*	*2,725.4*	21.84
Brazil	125.6	26.2	13.5	3.6	94.5	11.2	*274.7*	2.20
Total South and Central America	*302.2*	*148.6*	*28.2*	*5.0*	*165.7*	*15.6*	*665.3*	5.33
France	80.9	38.2	11.4	96.3	13.2	5.4	*245.4*	1.97
Germany	111.5	67.7	79.2	22.5	4.8	26.0	*311.7*	2.50
Greece	15.4	3.8	7.5	–	1.0	1.1	*28.8*	0.23
Italy	64.2	61.8	16.2	–	9.4	10.9	*162.5*	1.30
Kazakhstan	12.8	8.5	35.0	–	1.8	^	*58.1*	0.47
Russian Federation	147.5	374.6	93.9	40.3	37.8	0.1	*694.2*	5.56
Spain	63.8	28.2	19.3	13.9	4.6	14.9	*144.8*	1.16
Total Europe and Eurasia	*879.8*	*975.0*	*516.9*	*266.9*	*190.8*	*99.1*	*2,928.5*	23.47
Iran	89.6	140.5	0.9	0.3	2.9	^	*234.2*	1.88
Saudi Arabia	129.7	92.5	–	–	–	–	*222.2*	1.78
United Arab Emirates	32.6	56.6	–	–	–	^	*89.3*	0.72
Other Middle East countries	81.4	39.6	0.2	–	2.2	^	*123.5*	0.99
Total Middle East	*375.8*	*370.6*	*9.9*	*0.3*	*5.1*	*0.1*	*761.9*	6.11
Total Africa	*166.5*	*110.5*	*97.5*	*3.2*	*24.1*	*1.4*	*403.3*	3.23
Australia	46.7	22.9	49.3	–	4.1	2.8	*125.7*	1.01
China	483.7	129.5	1873.3	22.0	194.8	31.9	*2735.2*	21.92
India	171.6	49.1	298.3	7.5	26.2	10.9	*563.5*	4.52
Japan	218.2	105.1	124.4	4.1	18.3	8.2	*478.2*	3.83
South Korea	108.8	45.0	81.8	34.0	0.7	0.8	*271.1*	2.17
Total Asia-Pacific	*1389.4*	*562.5*	*2609.1*	*78.1*	*289.0*	*64.1*	*4992.2*	40.01
Total world	*4130.5*	*2987.1*	*3730.1*	*560.4*	*831.1*	*237.4*	*12476.6*	100.00

Primary energy comprises commercially traded fuels including modern renewables used to generate electricity. ^ Less than 0.05. Oil consumption is measured in million tonnes; other fuels are in million tonnes of oil equivalent
Source: BP Statistical World Energy Review (2013)

over this period. It is observed that modern renewable energy can substitute for fossil and nuclear fuels in four distinct markets: power generation, heating and cooling, transport fuels and rural/off-grid energy services (REN21 2013). It has been found that growth of renewables is highly dependent on many factors including government policy, business environment and investment climate of a country. It is evident that the global investment scenario for renewables has been improving over the last 8 years and total investment almost increased by sixfolds from $48.4 billion in 2004 to $296.7 billion in 2012 (REN 21 2013). A significant chunk of the above investment in 2012 was in the area of asset finance ($148.5 billion) and small distributed capacity ($80 billion), and only $9.6 billion was devoted for research and development activities. In terms of technology-based investment, solar fetched $140 billion followed by wind ($80 billion) and biomass ($8.6 billion).

In the Asia-Pacific region, Australia has been making a significant progress in terms of producing electricity from renewable sources. During the

Table 2 Primary and final energy from renewables

	Primary energy from renewables		Final energy from renewables	
Countries	Share (%) 2009–2010	Target (%)	Share (%) 2009–2010	Target (%) by 2020
Germany	9		12	18
France	6		13	23
India	7		4.9	–
Italy	11		12	17
Japan	6.9	10 % by 2020		15
Spain	13		15	20.8
Sweden	38		48	50
Norway	65			67.5
UK	4		3.8	15

Source: Compiled from various published sources

2012 calendar year, the contribution of renewable energy to Australia's electricity supply broke 10 % for the first time this century, producing more than 13 % of the total – powering the equivalent of almost 4.2 million homes – and wind energy contributed 26 % of renewables (Clean Energy Council 2012). Over the last 4 years, solar energy has been picking up very fast in Australia and generating close to 17,000 green jobs. Some recent media reports suggest that Australia may completely move to clean energy by 2030.

5 Role of Renewables in Green Economy

Renewable energy has been the point of discussion for the past decade and is becoming an increasingly important part of energy policy agenda for many countries including the USA and China. Some people like Taylor and Van Doren (2011) argue that renewable energy is quite literally the energy of yesterday and the progressive society abandoned "green" energy centuries ago for five very good reasons:

1. Green energy is diffuse, and it takes a tremendous amount of land and material to harness even a little bit of energy.
2. It is extremely costly compared to competing fossil fuel.
3. It is unreliable due to discontinuous availability of source of energy.
4. It is scarce in a sense that the real estate where those energies are reliably continuous and in economic proximity to rate payers is scarce.
5. Once the electricity is produced by the sun or wind, it cannot be stored because battery technology is not currently up to the task; hence it requires immediate consumption.

But in a world where fossil fuels are equally scarce resources, it is arguably a better option to have optimal mix of renewable and nonrenewable sources of energy rather than being heavily dependent on either one. Proponents of green energy argue that if the government can put a man on the moon, it can certainly make green energy economically attractive (Taylor and Van Doren 2011). In the recent times, many countries including the USA have been pushing for improving carbon footprint through renewable sources of energy. Of course to promote relatively costlier energy, government and administrations across the globe have been incentivising the producers and consumers through one or other forms of subsidies. Many scholars and opponents of renewable energy do argue that competitive energy pricing should be free from any form of subsidy. It is absolutely true in the long run, but in the short run adoptions of greener technologies require significant impetus for greater level of penetration. However, it should make economic sense in the long run. India has been seriously taking desirable steps to increase the share of green energy, most importantly renewable energy sources like wind and solar. Very recent developments suggest that India has been actively looking for international partners to provide techno-financial support for generation and distribution of renewable energy in the country. In September 2013, Germany committed financial and technical assistance to India for the green energy corridors, which includes financial assistance of 250 million euros as reduced interest loan. Technical assistance includes two million euros for an Indo-German energy programme and an additional two million euros for integration of renewable energies into the Indian electricity system (Singh 2013).

The government of India is strategically promoting renewables. The 12th Plan envisions installing 100 GW of new capacity of which 30 GW is projected to come from renewable energy sources, of which wind would account for 15 GW. According to the Planning Commission report, historically the Indian wind energy sector has met and occasionally exceeded its allocated target. During the 10th Plan period, the target set was of 1,500 MW, whereas the actual installations were 5,427 MW. Similarly, during the 11th Plan period, the revised target was for 9,000 MW and the actual installations were much higher at 10,260 MW (GWEC 2012). Globally, it is observed that there are several drivers driving growth of renewables. Some of the drivers are discussed here:

5.1 Environmental Sustainability

In many countries across the world electricity generation has been fossil fuel (i.e. coal, oil and gas) based. When fossil fuels are burned, they release carbon dioxide into the atmosphere that contributes to global warming. Incidentally, burning one ton of coal will produce between 1.5 and 3.5 tons of CO_2, depending on the carbon content of the coal, implying that a big coal power station produces 15–30 or more thousand tons of CO_2 daily. Global CO_2 emissions status suggests that few developed and developing countries contribute close to 50 % of CO_2 emissions. In 2012, China contributed 26.7 % of global CO_2 emissions followed by the USA (16.8 %), India (5.3 %) and the Russian Federation (4.9 %). In the case of India and China, CO_2 emissions grew by 6.9 % and 6 % (Table 3), respectively, over 2011. In rapidly growing economies like China and India, energy consumption is bound to go up and so are emissions of CO_2 and other greenhouse gases. As of June 30, 2013, about 69 % of total installed capacity for power generations in India was coal, oil and gas based. This is an indication that primary energy is driven by fossil fuel in India. In order to reduce greenhouse gases (GHG), India should invest in greener technologies like solar and wind. Wind power entails no direct GHG emissions and does not emit other pollutants (such as oxides of sulphur and nitrogen); additionally, it consumes no water (IEA 2013).

5.2 Uneven Spread of Fossil Fuel

Fossil fuels are not uniformly spread in terms of reserves. At the end of 2012 in terms of proved oil reserves, the Middle East had a share of 48.4 % followed by South and Central America (19.7 %), North America (13.2 %), Europe and Eurasia (8.4 %), Africa (7.8 %) and Asia-Pacific (2.5 %). However, in 2012 Asia-Pacific was the largest consuming region with a share of 33.6 % of total crude oil consumption. India consumed 4.2 % of total global crude oil consumption while India's share in terms of proven reserve is only 0.3 %. Another Asian giant, China is the second largest consumer of oil with only 1 % of total reserve. As far as coal production is concerned in 2012, China produced 1,825 Mtoe of coal followed by the USA (516 Mtoe), Australia (241 Mtoe), Indonesia (237 Mtoe) and India (229 Mtoe) (Table 4). Coal production in China has gone up drastically from 775 Mtoe in 2002 to 1825 Mtoe in 2012 (Table 4). In 2012, coal consumption (1875 Mtoe) was almost met by the domestic production. This is a very clear indication that China has been very much dependent on coal for energy production, mostly producing electricity. Coal production in the USA exceeded consumption (437.8 Mtoe) in 2012. A common perception is that India is rich with coal reserves and production can meet the demand. However, the popular perception is not true. India consumed 302 Mtoe of coal compared to production of 229 Mtoe having a deficit of 73 Mtoe, which means 24 % import dependence for coal. In 2012, coal consumption in India increased by 9.9 % over 2011 (270.6 Mtoe), and 8 % of global coal consumption came from India. Natural gas has a better geographical spread in terms of proved reserves compared to oil. At the end 2012, Iran had 18 % share with 33.6 trillion cubic metres (TCM) of proved natural gas reserve followed by the Russian Federation (32.9 TCM, 17.6 %), Qatar (25.1 TCM, 13.4 %), Turkmenistan

Table 3 Carbon dioxide emissions (in million tonnes)

	2003	2004	2005	2006	2007	2008	2009	2010	2011	**2012**	2011	Of total (2012)
USA	6,343	6,472	6,494	6,412	6,524	6,331	5,908	6,130	6,003	*5,786*	–3.9 %	16.8 %
Canada	631	629	636	628	641	631	590	611	624	*620*	–0.9 %	1.8 %
Mexico	401	413	440	448	449	448	455	459	474	*496*	4.3 %	1.4 %
Total North America	*7,375*	*7,514*	*7,570*	*7,489*	*7,614*	*7,410*	*6,953*	*7,200*	*7,101*	*6,902*	*–3.1 %*	*20.0 %*
Brazil	350	370	378	384	407	436	420	472	487	*500*	2.5 %	1.5 %
Total South and Central America	*996*	*1,054*	*1,083*	*1,128*	*1,177*	*1,229*	*1,213*	*1,300*	*1,347*	*1,388*	*2.8 %*	*4.0 %*
Germany	910	900	883	894	860	854	798	834	802	*815*	1.3 %	2.4 %
Russian Federation	1,605	1,605	1,593	1,660	1,661	1,688	1,581	1,645	1,709	*1,704*	–0.5 %	4.9 %
United Kingdom	595	602	604	605	588	590	540	558	518	*530*	2.2 %	1.5 %
Total Europe and Eurasia	*7,266*	*7,324*	*7,317*	*7,455*	*7,435*	*7,402*	*6,880*	*7,084*	*7,099*	*7,038*	*–1.1 %*	*20.4 %*
Iran	408	423	475	500	516	539	569	580	591	*608*	2.7 %	1.8 %
Saudi Arabia	378	410	421	439	458	498	521	564	577	*615*	6.4 %	1.8 %
Total Middle East	*1,339*	*1,427*	*1,525*	*1,569*	*1,631*	*1,763*	*1,827*	*1,924*	*1,971*	*2,063*	*4.4 %*	*6.0 %*
Total Africa	*898*	*949*	*976*	*986*	*1,036*	*1,097*	*1,085*	*1,116*	*1,109*	*1,157*	*4.0 %*	*3.4 %*
China	4,344	5,102	5,574	6,149	6,512	6,749	7,205	7,945	8,660	*9,208*	6.0 %	26.7 %
India	1,041	1,116	1,180	1,246	1,341	1,444	1,572	1,648	1,701	*1,823*	6.9 %	5.3 %
Japan	1,387	1,395	1,410	1,384	1,401	1,402	1,236	1,316	1,317	*1,409*	6.7 %	4.1 %
South Korea	580	591	602	606	640	653	662	714	754	*764*	1.0 %	2.2 %
Total Asia-Pacific	*9,381*	*10,336*	*10,983*	*11,694*	*12,304*	*12,641*	*13,142*	*14,216*	*15,117*	*15,919*	*5.0 %*	*46.2 %*
Total world	*27,254*	*28,603*	*29,453*	*30,320*	*31,197*	*31,540*	*31,100*	*32,840*	*33,743*	*34,466*	*1.9 %*	*100.0 %*

Source: BP Statistical Review (2013)

Table 4 List of top seven coal-producing countries in the world in 2012 (million tonnes oil equivalent (Mtoe))

	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
China	775	917	1,061	1,175	1,264	1,346	1,401	1,487	1,618	1,758	1,825
USA	570	554	572	580	595	588	597	541	551	556	516
Australia	184	189	197	206	211	217	224	232	236	231	241
Indonesia	64	70	81	94	119	133	148	158	169	217	237
India	138	144	156	162	170	181	196	211	218	216	229
Russian Federation	117	127	132	139	145	148	153	142	151	158	168
South Africa	124	134	137	138	138	140	142	141	145	142	147
Total world	2,402	2,573	2,781	2,942	3,101	3,211	3,324	3,354	3,543	3,759	3,845

Source: BP Statistical Energy Review (2013)

(17.5 TCM, 9.3 %), USA (8.5 TCM, 4.5 %), and Saudi Arabia (8.2 TCM, 4.4 %). India had 1.3 TCM with just 0.7 % of total proved reserve of 187.3 TCM.

5.3 Price Fluctuations of Fossil Fuel

Uneven spread of reserves and production of fossil fuel coupled with consumption driven by countries with limited fossil fuel lead to demand-supply imbalance. Asia-Pacific and the USA are the major demand centres of petroleum products, especially crude oil. OPEC countries have natural monopoly and can control production and to a great extent control prices of crude oil in the international market. Despite the USA being one of the largest producers of crude oil, it continued to be a net importer of crude oil. Crude oil being one of the largest traded commodities in the global market is subjected to price fluctuations. In the recent past the price fluctuations (Table 5) have been significant, and most of the times these fluctuations are unpredictable as price fluctuations are not driven by fundamentals. Many experts perceive that the sharp increases in the real price of oil, especially in 2007/2008, could be due to speculative trading and artificially maintaining low level production and supply by the producers. However, such price increases may not be explained by any one popular perception. Such price increases could be better

Table 5 Spot price of natural gas and crude oil

Date and year	Henry hub gulf coast natural gas spot price ($/MMBTU)	Cushing, OK WTI spot price FOB ($/bbl)	Europe Brent spot price FOB ($/bbl)
Jan 31, 2000	2.69	27.65	27.08
Jan 31, 2001	5.83	28.62	26.59
Jan 31, 2002	2.28	19.71	19.07
Jan 31, 2003	5.58	33.51	31.57
Jan 30, 2004	5.8	33.16	29.53
Jan 31, 2005	6.14	48.25	44.51
Jan 31, 2006	8.73	67.86	63.19
Jan 31, 2007	7.76	58.17	56.52
Jan 31, 2008	8.1	91.67	91.58
Jan 30, 2009	4.77	41.73	44.17
Jan 29, 2010	5.26	72.85	71.2
Jan 31, 2011	4.42	90.99	98.97
Jan 31, 2012	2.51	98.46	110.26
Jan 31, 2013	3.33	97.65	115.55

Source: Compiled from www.eia.gov
Spot price data on January 31 for 2004, 2009 and 2010 was not available, so the latest available data has been presented in the table

explained by a combination of factors (Dées et al. 2008; Hamilton 2008; Kilian and Murphy 2010) such as speculative trading, supply shocks, demand shocks and market structure (Breitenfellner et al. 2009). For example, business cycle factors were responsible for the bulk of the 1979/1980 oil price increase in conjunction with sharply rising speculative demand in 1979. Such fluctuations are bound to happen time and again like what happened in 1979, 1991 and 2008, and global consumers can do very little to control them. The policymakers should encourage energy conservation and promote development of alternate energy to reduce the impact of price fluctuations on local and global economy.

5.4 Availability Renewable Sources

Renewable sources of energy such as wind and solar are adequately available in various countries. Wind energy, like other power technologies based on renewable resources, is widely available throughout the world and can contribute to reduced energy import dependence (IEA 2013). Even though renewable resources are available, many countries are not seriously adopting renewable technologies due to the initial high cost. Even with a carbon price, emerging renewable energy technologies with good prospects for cost cuts could be given additional incentives to unlock their long-term potential (Philibert 2011).

5.5 Self-Sustainability and Energy Security

Self-sustainability is highly desirable; energy security is even critical to ensure growth at a sustained pace, especially in India. Energy security means different things to different people and country – security of oil and natural gas supply (Tanvi 2009), economic security, independence from imports, protection against price volatility (Pachauri 2005), affordability, accessibility and availability at competitive prices at all times and with a prescribed confidence level considering shocks and disruption that can be reasonably expected (Planning Commission, India, 2006).

It seems very difficult for a country like India to make energy available to common man at an affordable and competitive price while relying on over 70 % of imported energy. Balance of trade for crude stood at a deficit of 3,546.9 thousand barrels per day (Table 6).

With over a billion population and limited domestic fossil fuel reserves controlling global price, enhancing affordability and accessibility looks like a really difficult task. In such a scenario moving towards a global energy security system (GESS) seems a realistic option. As proposed by Kalicki and Goldwyn (2013), under GESS sharing technology and best practices, from carbon sequestration to large-scale power storage, and enhanced engagement with consumers and producers to forecast supply and demand, and respond to supply disruptions – precisely, quickly and effectively could be done. Such level of collaborative and mutually beneficial energy interdependence might reduce risk and lead towards safe, fuel-powered economic growth. For India, GESS could be one of the ways to improve energy security. Another one of the most important options is to harness renewable sources of energy for sustainable and long-term energy security.

5.6 Associated Costs

A very important difference between most renewable energy generation and fossil and nuclear power is the cost ratio between capital and operating costs (Heal 2009; REN21 2013). In case of coal-, nuclear- and natural gas-based power plants, the cost of fuel varies from moderate to very high compared to renewables like solar and wind where the fuel cost is zero. According to Heal (2009), fossil fuel power stations have significant fuel costs: a large coal-fired power station can use 10,000 tons of coal daily, costing between \$50 and \$100 per ton, so that fuel costs can be between half a million and a million dollars daily. If the cost of carbon is added, then the competitive price of coal-based power would significantly

Table 6 Oil: imports and exports in 2012 (thousand barrels daily)

	Crude imports	Product imports	Crude exports	Product exports	Balance of trade (crude)	Balance of trade (product)
USA	8,491	2,096	23	2,657	−8,468.6	561.5
Canada	514	211	2,437	619	1,923.1	407.8
Mexico	0.5	581	1,290	76	1,289.1	−504.5
South and Central America	392	1,411	3,143	691	2,750.7	−720.6
Europe	9,512	2,976	383	1,791	−9,128.8	−1,184.9
Former Soviet Union	0.5	114	6,049	2,548	6,048.6	2,433.8
Middle East	222	559	17,646	2,053	17,424.2	1,493.7
North Africa	186	312	2,139	465	1,952.4	152.9
West Africa	0.5	238	4,328	235	4,327.9	−3.2
East and Southern Africa	285	260	86	15	−199.2	−245.0
Australasia	575	379	272	164	−303.0	−214.6
China	5,433	1,729	26	538	−5,407.4	−1,191.3
India	3,547	323	0.5	1,349	−3,546.9	1,025.4
Japan	3,739	1,004	5.00	221	−3,733.9	−782.9
Singapore	948	2,016	12	1,479	−936.0	−536.3
Other Asia-Pacific	4,755	2,505	767	1,813	−3,987.8	−691.8
Total world	**38,599**	**16,715**	**38,599**	**16,715**	−0.0	–

Bunkers are not included as exports. Intra-area movements (e.g. between countries in Europe) are excluded
Source: BP Statistical Review of World Energy, June 2013

change. On the other hand, the major share of the cost of renewable power generation is capital invested upfront for the technology, project construction and grid connection. The marginal costs of most renewables (including hydro, geothermal, solar and wind power) are low and often prevail over conventional power generation on spot markets, thereby reducing the economic viability of marginal-cost-based generation (REN21 2013). Building a coal-based power plant with relatively low initial capital cost and high continuous fuel cost means for another 30 years the consumer is going pay a higher price in the future. Building a wind power generation facility means investing upfront and ripping benefit for 25–40 years. In addition to this, the external costs of renewables are far less than those of fossil fuels (Burtraw and Krupnick 2012), particularly with respect to emissions of greenhouse gases (Heal 2009) and other environmental and health hazards. Nordhaus (2009) has an estimate of the social cost of CO_2 emissions that is about $8 per ton, and Stern (2006) estimates an order of magnitude greater at $85 per ton. Heal (2009) suggests that Stern's estimate seems to be closer to reality but not free from ambiguity. The European Commission conducts studies on social cost, and reports suggest that external costs of renewables like solar and wind are as low as 0–0.6 c/kwh compared to nonrenewables as high as 11 c/kwh. It could be safely argued that considering high associated costs of fossil fuel the renewables are a safer and better bet for future socioeconomic and environmental progress.

5.7 Geopolitical Dynamics

In the place of the USA and the European Union, Asia is now becoming the predominant buyer of Persian Gulf oil and gas, primarily driven by strong demand growth from China and India. Consequently, one of the foundations of US interests in the gulf is weakening as global oil flows shift dramatically towards Asia (Herberg 2012) and the role of India and China is becoming very crucial in global energy geopolitics. India could derive desirable benefits by strengthening relations with countries like Iran, Iraq and Saudi Arabia. China has been building very strong relationships with many countries in the Middle East and North Africa (MENA) region. India needs to build mutually beneficial relationships in the MENA region to reduce geopolitical risk associated with sourcing of fossil fuel especially crude oil and natural gas. Overdependence on any outside source for one or more forms of energy means supply and demand are vulnerable to external shocks arising out of geopolitical tension or man-made disasters like terrorist attacks. Any such undesirable event may disturb supply causing energy imbalance in the country. In order to reduce vulnerability and ensure higher level of supply security, optimal energy mix with higher contribution from renewables is highly desirable. Global geopolitical dynamics certainly need to be factored in while developing optimal energy mix in a country like India. Unfavourable geopolitical dynamics could be one of the major drivers to harness domestic energy resources, especially the renewables.

6 Role of Green Fuel in Green Economy

If green economy is to become a reality, most of the countries having rich renewable resources must move to renewable energy at the earliest. Sectors that are important constituents of green economy are buildings (residential, commercial and industrial), manufacturing and transport. Buildings account for almost a third of final energy consumption globally and are an equally important source of CO_2 emissions. According to EIA (2011), space heating and cooling as well as hot water are estimated to account for roughly half of global energy consumption in buildings. Globally, the industrial sector consumes 51 % of total energy followed by the transportation sector (20 %), residential sector (18 %) and commercial sector (12 %). The industrial and transportation sectors are clear drivers of energy consumption and should transition from fossil fuel to green fuel. Transition to green fuel not only improves environmental condition but creates additional economic and trade activities also. A recent report by UNEP suggests that the global market in low-carbon and energy-efficient technologies, which include renewable energy supply products, is projected to nearly triple to US$ 2.2 trillion by 2020. It has been observed that over the last decade the emerging economies like China and India have significantly improved their exports of renewable energy equipment such as solar panels, wind turbines and solar water heaters. Apart from exporting components of new technologies, various developing countries are also expanding their potential to export electricity from renewable sources. Such trade activities would certainly improve balance of trade and create healthy relationships with neighbouring importing countries. For example, Nepal has an estimated 43,000 MW hydropower generation capacity, but the actual production is only 650 MW (Kaul 2012). Nepal recently signed an agreement with China to develop a hydropower plant (750 MW) with an investment support of $1.6 billion from China. Power produced from the new plant will improve electricity supply in Nepal, and surplus would be supplied to China. India seems to be geared up for further mutual agreement for power trading, and very recently the chargé d'affaire at the Indian Embassy in Nepal Jaideep Mazumdar said, "We are positive about signing the agreement as it would benefit northern Indian states, which are now depending on fuels like coal that is polluting the environment" (The Himalyan News Service 2013). India and Bhutan have already entered power trade agreements in the 1980s. Bhutan is exporting approximately 450 MW of hydropower towards India and earning inconvertible Indian currency, thereby boosting the pace of economic growth of Bhutan (Sigdel 2007). It is suggested that a regional electricity grid in Asia should be developed to capitalise on power shortage and surplus. More specifically to share surplus electricity produced from renewables. Renewables are considered to be the future of energy and a global driver of green economy. Green fuels will drive green industries, green production, green buildings, green transport, green products and green job market. Some of the important forms of green energy such as solar, wind, biomass, hydro and natural gas that are likely to be important drivers of green economy are discussed here.

6.1 Solar Energy

Green economy is bound to be driven by green fuel in the future, especially solar energy likely to play a pivotal role for many green economies. In the recent past, employment growth rate suffered significantly even in the most advanced countries like the USA and developing countries like India. Solar energy is not only going to improve domestic energy supply scenario in many countries like India, China and the USA, but also create huge job opportunities in power generation, equipment manufacturing and allied sectors. Such developments are going to improve employment, social welfare and living standards in countries like India. China has been making a steady impact on export of solar panels and cells. In 2012, in the solar sector, China has exported over US$ 10 billion worth of solar panels and cells, almost 80 times the value it exported only 10 years earlier. Similarly in India there is a thrust on developing manufacturing capabilities within the country and easing of import restrictions for efficient deployment of advanced technologies like solar PV. Developing homegrown technologies and manufacturing facilities would be helpful for the growth of local economy, promote entrepreneurial venture and create employment and moreover build global reputation.

The Worldwide Solar Water Heating System (SWHS) has been attracting the attention of the policymakers especially due to its high potential to reduce electricity consumption in domestic, commercial and industrial segments and consequent emissions reduction. In countries like India having abundant solar energy, available almost around the year could be used for SWHS. Several cities across India even suffer from acute power shortage, and residents don’t get electricity for heating purposes. Many households use subsidised liquefied petroleum gas (LPG) for water heating, and it does impact the economic environment. A better solution of water heating could be SWHS. However, the high initial cost of the SWHS has been found to be the major hurdle for large-scale deployment of SWHS. To reduce customer inertia and increase adoption of SWHS, the government devises and implements desirable customer-friendly schemes liking giving financial subsidy for installing SWHS. Such incentives help consumers to adopt new and renewable technologies at an affordable price point. Another area which could take solar revolution to the next level is rural lighting and cooking. Renewables like solar could be used to improve lighting conditions in rural India. One such initiative has been taken by the Energy Resource Institute (TERI). In 2008, TERI initiated ‘Lighting a Billion Lives (LaBL)’ campaign. Under the initiative, about 100,000 solar lanterns have been provided to rural households, benefiting nearly 1.3 million lives across the country. Similarly, the initiative on cooking stoves has reached out to close to 2,131 households in Uttar Pradesh (Times News Network 2013). On average, India has 300 sunny days per year and receives an average hourly radiation of 200 MW/km^2. The Jawaharlal Nehru National Solar Mission (JNNSM) has set a target of achieving at least 20,000 MW of grid-connected solar power by 2022, and this could put India among the leading solar countries in the world, not only in total installed solar capacity but also in manufacturing components and technology research and development.

6.2 Wind Energy

In 2012, the cumulative installed wind turbine capacity in the world reached 284,237 MW from 47,935 MW in 2004. China (26.5 %) has been leading the way in terms of capacity addition followed by the USA (21.2 %), Germany (11 %), Spain (7.9 %) and India (6.5 %). About 73 % of capacity addition in 2012 has been contributed by the top five countries (Table 7). Strong growth of wind energy albeit from a lower base is a very clear indicator that leading energy-consuming countries are moving very fast towards green energy. For example, the USA added 13 GW of wind energy in 2012–2013 representing capacity to power more than 15 million homes each year, wind becoming the fastest-growing source of power in the USA and estimated to employ over 80,000 American workers. The tremendous growth of the wind industry in the USA over the past few

Table 7 Cumulative installed wind turbine capacity (MW)

	2004	2005	2006	2007	2008	2009	2010	2011	2012	CAGR (%)	Share in 2012 (%)
Total World	**47,935**	**59,186**	**74,089**	**94,091**	**121,883**	**160,148**	**197,873**	**239,125**	**284,237**	21.9	100.0
China	769	1,264	2,588	5,875	12,121	25,853	44,781	62,412	**75,372**	66.4	26.5
US	6,750	9,181	11,635	16,879	25,237	35,159	40,274	47,084	**60,208**	27.5	21.2
Germany	16,623	18,390	20,579	22,194	23,826	25,703	27,191	29,071	**31,315**	7.3	11.0
Spain	8,462	10,013	11,595	15,155	16,699	19,160	19,850	21,239	**22,362**	11.4	7.9
India	3,000	4,430	6,270	7,845	9,655	10,926	13,065	16,179	**18,420**	22.3	6.5
United Kingdom	889	1,336	1,955	2,477	3,406	4,424	5,378	6,488	**8,871**	29.1	3.1
Italy	1,261	1,713	2,118	2,721	3,731	4,845	5,793	6,733	**7,998**	22.8	2.8
France	386	775	1,585	2,471	3,671	4,775	5,961	6,836	**7,593**	39.2	2.7
Canada	444	683	1,459	1,845	2,371	3,321	4,011	5,278	**6,214**	34.1	2.2
Portugal	585	1,087	1,716	2,150	2,829	3,474	3,837	4,214	**4,363**	25.0	1.5
Denmark	3,083	3,087	3,101	3,088	3,159	3,408	3,805	3,927	**4,137**	3.3	1.5
Australia	421	717	796	972	1,587	1,886	2,084	2,476	**2,834**	23.6	1.0
Japan	991	1,159	1,457	1,681	2,033	2,208	2,429	2,595	**2,673**	11.7	0.9
Brazil	29	29	237	247	341	606	931	1,431	**2,508**	64.1	0.9

Source: Compile from BP Statistical Review 2013 years has been due to consistent policy devised and implemented by President Obama's administration to ensure that America remains a leader in clean energy innovation. In 2012, the USA invested $ 25 billion in wind energy and committed to continue investment in the future. According to Wiser and Bollinger (2013) from the US Department of Energy, regardless of future uncertainties, wind power capacity additions over the past several years have put the USA on an early trajectory that may lead to 20 % of the nation's electricity demand coming from wind energy by 2030. In order to educate, engage and enable critical stakeholders to make informed decisions about wind energy, the US Department of Energy designed 'Wind Program' and continuously organised events like seminars, webinars, etc. to achieve the objectives. According to new statistics from the China Electricity Council, China's wind power production actually increased more than coal power production for the first time ever in 2012 (Shuo 2013). Thermal power use, which is predominantly coal, grew by only about 0.3 % in China during 2012, an addition of roughly 12 terawatt hours (TWh) more electricity. In contrast, wind power production expanded by about 26 TWh. This rapid expansion brings the total amount of wind power production in China to 100 TWh, surpassing China's 98 TWh of nuclear power. In 2012, the total cumulative installed wind capacity of India stood at 18.4 GW with 6.5 % of global capacity. Leaders of wind energy like China and the USA are far ahead of India. China has installed wind capacity which is four times higher than India. This is a clear indicator that China is really diversifying the energy mix at a faster rate. It is advisable that India should strive hard to learn from the leaders to achieve the objective of 33 GW of installed capacity by 2017.

In India, wind technology was found to be relatively cost effective compared to solar PV and solar thermal, biomass and biogas (Table 8). The government of India (GoI) offers the following important fiscal and promotional incentives (IWEA 2013) to encourage production, distribution and consumption of wind energy in India:

1. Concessional import duty on specified wind turbine parts
2. Eighty percent accelerated depreciation in the first year
3. Excise duty reliefs
4. Loans through the Indian Renewable Energy Development Agency (IREDA)
5. Income tax holiday applicable to wind power as in the case of power projects

Table 8 Renewable energy projects, capital cost and levelised tariff for FY 2013–2014 in India

Technology	Capital cost (lakhs/MW)	Tariff (INR/KWh)
Wind energy	595.99	3.6 to 5.7
Small hydro projects		
A. Himachal Pradesh, Uttarakhand and northeastern states (less than 5 MW)	798.11	4.02
B. Himachal Pradesh, Uttarakhand and northeastern states (5 MW to 25 MW)	725.55	4.02
C. Other states (below 5 MW)	621.9	4.74
D. Other states (5 MW to 25 MW)	570.08	4.01
Biomass power projects	462.33	5.4 to 6.1
Non-fossil-fuel-based co-generation power projects	436.36	4.8 to 5.96
Solar PV power projects	800	7.9
Solar thermal power projects	1,200	10.7
Biomass gasifier power projects	421.42	5.8 to 6.6
Biogas power projects	842.85	6.7

Source: Green Peace Report on "Powering Ahead with Renewables, Leaders and Laggards" (2013)

With clear intention of massive improvement in wind energy production, distribution and consumption, GoI has been very proactively making concentrated efforts. In order to achieve the intended result of increasing share of green energy, the government has been making policies to:

1. Broaden the investor base
2. Incentivise actual generation with the help of a generation-/outcome-based incentive
3. Facilitate entry of large independent power producers (IPPs) and foreign direct investors (FDI) to the wind power sector

Recently in September 4, 2013, GoI notified the extension of the scheme for continuation of generation-based incentive (GBI) for grid interactive wind power projects during the 12th Plan period. According to the notification under the scheme, a GBI will be provided to wind electricity producers at Rs. 0.50 per unit of electricity fed into the grid for a period not less than 4 years and a maximum period of 10 years with a cap of Rs. 100 lakhs per MW. The total disbursement in a year will not exceed one fourth of the maximum limit of the incentive, i.e. Rs. 25.00 lakhs per MW during the first 4 years. The GBI scheme will be applicable for the entire 12th Plan period having a target of 15,000 MW. The GBI scheme would be implemented in parallel with the existing fiscal incentives for grid-connected wind power projects. The GBI will cover grid-connected generation from wind power projects set up for sale of electricity to grid at a tariff fixed by State Electricity Regulatory Commissions (SERC) and/or the state government and also include captive wind power projects including group captive but exclude third party sale (viz. merchant power plants). Such incentives would certainly encourage the investors, producers and consumers of green energy. Incentive schemes like GBI would help building huge confidence among the investors and producers as such confidence-building measures might ensure some unviable projects to be commercially viable.

6.3 Biomass Energy

Studies sponsored by the Ministry of New Renewable Energy (MNRE) has estimated surplus biomass availability at about 120–150 million metric tonnes per annum (MMTPA) covering agricultural and forestry residues with a potential of 18,000 MW power generation in India. As of March 31, 2011, the total installed biomass power/co-generation capacity in India reached 2664.63 MW from 483.93 MW in 2003. Uttar Pradesh leads with 592.50 MW followed by Tamil Nadu (488.20 MW), Maharashtra (403 MW), Karnataka (365.18 MW) and Andhra Pradesh (363.25 MW). Energy generation from biomass has been actively promoted with appropriate incentive schemes in India. The government offers fiscal incentives such as 10-year income tax holidays, customs and excise duty exemptions for machinery and equipment and sales tax exemptions in some states. Fiscal incentives like 80 % depreciation in the very first year could be claimed for the following equipment required for co-generation systems:

1. Back pressure, pass-out, controlled extraction, extraction-cum-condensing turbine for co-generation with pressure boilers
2. Vapour absorption refrigeration systems
3. Organic rankine cycle power systems
4. Low-inlet-pressure small steam turbines

Similarly, biogas is another clean and low-carbon technology-driven fuel. Through efficient management and conversion of organic wastes, clean renewable biogas along with bio-fertiliser could be produced. Biogas has a great potential to leverage sustainable livelihood, especially in rural India. Energy produced from biogas provides a solution to unavailability of cooking fuel and electricity in rural India. Based on the availability of cattle dung alone from about 304 million cattle, there is an estimated potential to produce about 18,240 million cubic metres of biogas annually. Biogas could complement with other renewables like solar to improve affordability, accessibility and availability of energy to all citizens, especially in rural India. This could really change rural livelihood and socioeconomic outlook in big ways.

6.4 Hydro Power

According to the RNE21 (2013) report, an estimated 30 GW of new hydropower capacity came online in 2012, increasing global installed capacity by about 3 % to an estimated 990 GW. The top countries for hydro capacity are China, Brazil, the USA, Canada, and Russia, which together account for 52 % of total installed capacity. Globally, hydropower generated an estimated 3,700 TWh of electricity during 2012, including approximately 864 TWh in China, followed by Brazil (441 TWh), Canada (376 TWh), the USA (277 TWh), Russia (155 TWh), Norway

(143 TWh) and India (>116 TWh). In India hydropower contributes about 18 % of total installed capacity (Table 9) of power. The total hydroelectric power potential in the country is assessed at about 150,000 MW (see EIA). The potential of small hydropow er projects is estimated at about 15,000 MW. Currently, the country is able to harness about 26 % of the total potential and needs significant strategic efforts to convert potentials into reality.

One way of capitalising on the potential hydropower is to actively encourage and give incentives to investors to invest in small hydro projects. For promoting small hydro projects, the Ministry of New and Renewable Energy (MNRE) offers financial incentives for states in the north-eastern region, Jammu and Kashmir, Himachal Pradesh and Uttarakhand (Special Category States) and other states. Both government sector and private sector companies are entitled for such benefits. The government gives financial incentive of Rs 20,000 per kW (up to thousand kW) and Rs 2.00 crore for the first MW with Rs. 30 lakhs for each additional MW (up to 25 MW). Plain and other regions of all other states, the above-mentioned incentives are reduced to Rs.12,000 per kW and Rs.1.2 crore with an addition of Rs. 20 lakhs for each additional MW up to 25 MW (Table 10).

Table 9 Total installed capacity (MW) in India as of June 30, 2013

Coal	132,288.39	58.59
Gas	20,359.85	9.02
Oil	1,199.75	0.53
Hydro (renewable)	39,623.40	17.55
Nuclear	4,780.00	2.12
RES[a] (MNRE)	27,541.71	12.20
Total	225,793.10	100.00

SHP small hydro project; *BG* biomass gasifier; *BP* biomass power; *U&I* urban and industrial waste power; *RES* renewable energy sources

[a] Renewable energy sources (RES) include SHP, BG, BP, U&I and wind energy

Source: http://www.powermin.nic.in/indian_electricity_scenario/introduction.htm

Hydroelectric power is essentially a clean and renewable form of energy. However, the construction of hydroelectric power plants, especially those of very large scale, poses a few environmental problems like construction of dam and downstream water environment. Hence, the government needs to take necessary care and precautionary measures to protect the interest of local people, environment and climate conditions.

6.5 Natural Gas

Natural gas is not a renewable form of energy but it is considered to be much greener fuel compared to coal and liquid petroleum. Although India is not self-sufficient in terms of meeting current natural gas demand, strategic planning to ensure better procurement and development of gas infrastructure like re-gasification terminal, transmission pipeline and city gas distribution system could ensure higher level of availability and accessibility. Some of the countries in Europe having better accessibility to gas, especially Russian piped natural gas (PNG), have developed

Table 10 Incentive for small hydropower projects up to 25 MW

	Private, co-operative, joint sector, etc.		Government, state and public sector	
	Up to 1,000 KW	Above 1 MW and up to 25 MW	Up to 1,000 kW	Above 1 MW and up to 25 MW
N.E. region, J and K, Himachal Pradesh and Uttarakhand (Special Category States)	Rs 20,000 per kW (up to 1,000 kW)	Rs 2.00 crore for first MW + Rs. 30 lakhs for each additional MW	Rs 50,000 per kW	Rs 5.00 crore for first MW + Rs. 50 lakhs for each additional MW
Plain and other regions of all other states	Rs 12,000 per kW	Rs 1.20 crore for first MW + Rs. 20 lakhs for each additional MW	Rs 25,000 per kW	Rs 2.50 crore for first MW + Rs. 40 lakhs for each additional MW

Source: http://www.ireda.gov.in/forms/contentpage.aspx?lid=1,340 excellent gas-based infrastructure. On the other hand, countries like the USA, Russia, Qatar and Iran are rich with natural gas reserves and are natural users of their own resources. On the contrary, countries like Japan, Spain, China and India are relying on heavily imported gases either in the form of LNG or PNG. A glance at global consumers of natural gas in 2012 suggests that the USA (722.1 BCM, 21.9 % of global consumption), the Russian Federation (416.2 BCM, 12.5 %), Iran (156.1 BCM, 4.7 %), China (143.8 BCM, 4.3 %) and Japan (116.7 BCM, 3.5 %) are the leading consumers. In 2012, India consumed 54.6 BCM (1.6 %) of natural gas and produced only 40.2 BCM (1.2 % of global production). Low level of production happened due to a drastic fall in gas production from gas fields in the KG-D6 block in the east coast of India. The highest production achieved 62–63 million standard cubic metres per day (MMSCMD) in August 2010 and reached as low as 17 MMSCMD in October 2013. Countries like Japan, Spain and Pakistan don't have adequate natural gas reserves but have significant gas infrastructure in place. India needs to promote natural gas as a competing fuel. Of course, to a large extent India continues to import natural gas from the MENA region to meet the demand. Unlike China, India doesn't import gas through pipelines; rather it relies on liquefied natural gas (LNG) import. Qatar and Nigeria are the primary sources of LNG import for India. Recently, Australia and the USA emerged as new sources of LNG import. LNG import from Australia could prove relatively costly compared to Qatar. Over the last decade, India has been able to develop fairly good LNG infrastructure having LNG receiving terminal and re-gasification facility at Dahej (10 MMTPA), Hazira (3.5 MMTPA), Dabhol (5 MMTPA) and Kochi (4 MMTPA). The LNG re-gasification facilities are planned in east coast (5 MMTPA), Mundra (5 MMTPA), Ennore (5 MMTPA), west coast (2.5 MMTPA), Pipavav (12.5 MMPTA), Jamnagar (5 MMPTA) and Dhamara (5 MMTPA) and all planned facilities could come up by 2019–2020. In order to secure LNG supply, long-term contracts have been the preferred option along with short term and spot buying. For example, the Gas Authority of India Limited (GAIL) – a state-owned gas company – has been successful in securing LNG under long-term agreements from suppliers like Sabine Pass (USA), Dominion Cove (USA) as well as Gazprom (Russia). GAIL's imported volumes are from the US Sabine Pass Project (3.5 MMTPA); US Dominion Cove Point (2.3 MMTPA); and Gazprom, Shtokman LNG project (2.5 MMTPA) for which supplies are expected to commence during 2018–2020. Further discussions are underway with other suppliers also from countries like the USA, Qatar, Algeria, Mozambique and Nigeria for securing additional volumes. GAIL bought 20 cargoes in the last fiscal year and would be buying some 34 LNG cargoes in the current fiscal year and 34–35 next fiscal (Press Trust of India 2013). In addition to the relatively expensive LNG import, India needs to build a transnational gas pipeline for importing natural gas from gas-rich countries like Iran, Turkmenistan and Myanmar. Recently, the Comptroller and Auditor General (CAG) raised queries over inadequate progress made in the execution of transnational pipeline projects, including the Iran-Pakistan-India (IPI) pipeline, Turkmenistan-Afghanistan-Pakistan-India (TAPI) gas pipeline and the almost-defunct Myanmar-Bangladesh-India gas pipeline (The Hindu 2013). Some progress has been made in the recent past (in 2012); India, Pakistan, Afghanistan and Turkmenistan signed an agreement for building the TAPI pipeline to carry 90 MMSCMD of gas for a 30-year period and is likely to become operational by 2018. India and Pakistan would get 38 MMSCMD each, while the remaining 14 MMSCMD will be supplied to Afghanistan (The Hindu 2012). The countries are going to be immensely benefited from the TAPI deal, especially India and Pakistan. Pakistan and Afghanistan are not only going to receive gas but also a transit fee of 50 cents/MMBTU. The contract price of TAPI gas is linked to a formula which contains indices based on fuel basket and other indices which are not as volatile as crude oil, so it would give supply security and price stability. Another pipeline project that is believed to be very crucial for gas market development in India is the India-Pakistan-Iran

(IPI) pipeline. That has been stalled for quite some time now due to pure geopolitical reasons.

Transnational pipelines are difficult and complex ventures since they involve different countries with different economic and political interests. It is well documented that the transnational pipelines are difficult to execute as they pass through difficult terrain and politically and environmentally sensitive areas and hence require mobilisation of huge financial and technological resources. It is highly desirable that India should pursue transnational pipeline deals with gas resource-rich countries in the region. This would de-risk supply security to a greater extent and add to development of a green economy.

With the current domestic gas supply and imported LNG, over 50 Indian cities are getting natural gas supply. Essentially consumers in domestic, industrial and commercial segments are getting piped natural gas (PNG), and the transport sector is getting compressed natural gas (CNG). Over 2.3 million domestic, 17,000 commercial and 5,000 industrial consumers (Table 11) are using natural gas in the country. The industrial customers that are spread across industries like ceramics, power, textile, steel, packaging, pharmaceutical, petrochemical, refinery and cement do prefer natural gas as fuel over other existing alternatives (Kar and Sahu 2012). Many states like Delhi, Gujarat and Maharashtra (Table 12) have taken a lead in terms of CNG-driven transport system. States like Delhi have got huge benefits like a drop in pollution level from CNG-driven transport system.

7 Pricing of Renewable Energy

Pricing of renewable energy seems to be an important issue in the context of renewable energy production, distribution and consumption. Of course, in many countries the renewables have not achieved grid parity because of higher cost of technology, lack of grid integration and other operational constraints. Energy from nonrenewables or fossil fuel was found to be comparatively cheaper, accessible and available. However, the pricing of fossil fuel doesn't include the environmental externalities. Many environment-conscious researchers argue in favour of including all environmental and social externalities for calculation pricing of fossil fuel-based energy. Even without that in some countries the renewables have achieved grid parity with other available sources of nonrenewable energy. As reported by Paton (2013), electricity generated from new wind farms costs about ~$80 ($84) for each megawatt per hour (MWh) produced, while electricity from new coal plants costs about $95/MWh and electricity from new natural gas plants costs about $100/MWh. The above prices are excluding of Australia's carbon tax ($23.5 per tonne of carbon emissions); if included, it makes the real market price of coal power about $146/MWh and natural gas about $119/MWh (EESI 2013). In India, renewables have achieved parity with electricity produced from diesel-based generators and gas (imported spot LNG)-based power plants. However, renewables have a long way to go to achieve grid parity with coal-fired or domestic gas-based power plants. Renewables pricing in India is receiving much needed attention, and the government at the centre and at the state level through their electricity commissions is trying to ensure a level playing field for the renewable power producers. The power distribution companies at the state level are required to purchase a mandatory percentage of electricity from renewable sources. Through this the regulatory commissions are trying to ensure off-taker of renewable power from the producers at a fairly competitive price set by the regulators (Table 13). Such regulatory interventions are not only desirable but necessary to promote green energy. Regulatory enforcement could change the market demand for renewables and possibly help fasten adoption and early market development. Large-scale production of renewables would bring down the cost of production, and efficient grid integration could drive down cost of transmission and distribution. In the long run, efficient production and transmission could ensure higher return on investment and finally attract more investment for renewables. This could bring renewable price parity with existing options for the final consumer. In order for

Table 11 PNG connection status (as of March 31, 2013)

State	City covered	Company	Number of Domestic PNG connections	Domestic PNG share (%)	No. Comm. PNG connections	Comm. PNG share (%)	No. Ind. PNG connections	Ind. PNG share (%)
Delhi	Delhi, NOIDA, Gr. NOIDA, Ghaziabad	IGL	410,000	18.14	1,370	7.97	570	10.59
Maharashtra	Mumbai, Thane, Mira-Bhayandar, Navi Mumbai, Pune, Kalyan, Ambernath, Panvel, Bhiwandi	MGL, MNGL	647,790	28.67	1,990	11.58	98	1.82
Gujarat	Ahmedabad, Baroda, Surat, Ankleshwar, Himmatnagar, Gandhinagar, Rajkot, Vapi, Morbi, Than	GSPC, Sabarmati Gas, Gujarat Gas, HPCL, VMSS, Adani Gas	1,144,424	50.65	12,693	73.85	3,686	68.51
Uttar Pradesh	Agra, Kanpur, Bareilly, Lucknow	Green Gas Ltd. (Lucknow), CUGL (Kanpur)	7,090	0.31	55	0.32	430	7.99
Tripura	Agartala	TNGCL	11,431	0.51	256	1.49	41	0.76
Madhya Pradesh	Dewas, Indore, Ujjain, Gwalior	GAIL Gas, AGL	1,775	0.08	6	0.03	49	0.91
Rajasthan	Kota	GAIL Gas	177	0.01	0	0.00	16	0.30
Assam	Tinsukia, Dibrugarh, Sibsagar, Jorhat	Assam Gas Co. Ltd.	23,632	1.05	759	4.42	366	6.80
Andhra Pradesh	Kakinada, Hyderabad, Vijayawada, Rajahmundry	BGL	1,802	0.08	15	0.09	1	0.02
Haryana	Sonepat, Gurgaon, Faridabad	GAIL Gas, Adani Gas, Haryana City Gas	11,508	0.51	43	0.25	123	2.29
Total			2,259,629	100	17,187	100	5,380	100

Source: PPAC, IGL, GSPC, MGL (Comm, commercial; Ind, industrial)

Table 12 Status of CNG stations and vehicles (as of March 31, 2013)

State	Company name	No. of CNG stations	Share (%)	No. of CNG vehicles	Share (%)
Gujarat	GAIL Gas/Adani Energy/Gujarat Gas, GSPC, GGCL, SGL, HPCL	313	34.51	638,422	34.08
Delhi/NOIDA, Gr. NOIDA/Ghaziabad	Indraprastha Gas (IGL), New Delhi	290	31.97	720,000	38.43
Maharashtra	Mahanagar Gas Ltd. (MGL), Mumbai; MNGL, Pune	203	22.38	334,810	17.87
Andhra Pradesh	Bhagyanagar Gas Ltd. (BGL) Hyderabad	29	3.20	19,958	1.07
Rajasthan	GAIL Gas	2	0.22	1,085	0.06
Uttar Pradesh	Green Gas Ltd. (Lucknow); CUGL (Kanpur)	30	3.31	56,857	3.03
Tripura	Tripura Natural Gas Co. Ltd. (TNGCL), Agartala	3	0.33	4,682	0.25
Madhya Pradesh	Avantika Gas (Indore)/GAIL Gas Ltd.	16	1.76	10,878	0.58
Haryana	Haryana City Gas Ltd.	14	1.54	85,560	4.57
West Bengal	GEECL	7	0.77	1,201	0.06
All India		**907**	100.00	**1,873,453**	100.00

Source: PPAC, GSPC, IGL

renewable price parity with fossil fuel-based power to happen, the government needs to reduce the subsidy component on fossil-based power and divert the subsidy to renewables to drive renewable production and consumption. It is visible that the government offering appropriate incentives and the regulatory bodies setting attractive tariff for renewable energy producers (Table 13 and 14).

7.1 Green Economy in India

Visions of a green economy have assumed different meanings and definitions from country to country depending on national strategies and priorities (Govindan 2012). While addressing a meeting organised for the Executive Director for UNEP on June 3, 2011, Tishya Chatterjee, the Secretary of the Ministry of Environment and Forests, Government of India, acknowledged that there is a strong need for moving towards a green economy in India. There are reasonable and compelling arguments in favour of a green economy in India. Some of these are as follows:

- A green economy is a way forward truly to improve human well-being and achieve the millennium development goals.
- It promotes equity, not just in the nature of the common but differentiated responsibilities of the Rio Principles, but also as an expanded policy space for diversified sustainable development.
- It provides a win-win economic-environmental model that ensures that economic and environmental synergies prevail over trade-offs.

The green economy is essentially an inclusive concept comprising the economic, social and environmental pillars of growth (Chatterjee 2011). Developing countries like India can continue to pursue its development agenda with sustainable means. Of course, the developed nations have to take the lead in terms of changing production and consumption pattern and show the developing world the path of sustainability. This can be essentially done

Table 13 Wind energy tariffs across states in India

State	RPS (%) specified	Tariffs fixed by commissions in INR per kWh	Validity of tariff (year)	Charges for captive users
Tamil Nadu	14 %	3.39 (fixed)	20	10 % (includes 5 % for banking if applicable)
Karnataka	10 %	4.2[a]	10	2–5 %
Maharashtra	6 %	5.07 (Wind Zone 1)[b] 4.41 (Wind Zone 2)[b] 3.75 (Wind Zone 3)[b] 3.38 (Wind Zone 4)[b]	13	Actual open access (OA) charges
Rajasthan	7.45 %	3.83 for Jaipur, Jodhpur and Barmer district 4.03 for rest of Rajasthan	20	50 % of normal OA charges
Andhra Pradesh	5 %	3.5	10	Actual OA charges
Madhya Pradesh	10 %	4.35	25	2 % plus transmission charge
Kerala	3 %	3.14 (fixed)	20	5 %
West Bengal	4–6.8 %	4.00 (fixed, to be used as a cap)	Flexible	2 %
Gujarat	4.50 %	3.56	25	4 %
Haryana	10 %	4.08 (with 1.5 % escalation per year)	5	2 %

[a] Revised price by KERC on October 10, 2013

[b] (Wind Zone 1 – annual mean WPD of 200–250 w/m^2)

[b] (Wind Zone 2 – annual mean WPD of 250–300 w/m^2)

[b] (Wind Zone 3 – annual mean WPD of 300–350 w/m^2)

[b] (Wind Zone 4 – annual mean WPD > 400 w/m^2)

Source: http://www.inwea.org/tariffs.htm (accessed on October 21, 2013)

through technology transfer, finance and reforms to global and financial structures. It is desirable that the developing countries like India realise that green economy projects and programmes are co-beneficial, bringing in revenues from both environmental and economic investments.

Indians are found to be enterprising and there is huge investment potential in the country. However, money does not flow into enhancing and accounting for ecosystem services except as a social responsibility which off late has been legally enforced. Voluntary involvement in socio-environmental protection activities seems to be less aspiring for corporate India. Of course few corporate houses have been doing the desirable religiously for years. But most of them are far away from discharging the socio-environmental responsibilities. One major explanation for low voluntary involvement could be that "all that is environmental is Government led and Government managed with all the attendant framework limitations" (Chatterjee 2011). It seems that the relationship of the private sector with the environment is on a regulatory platform, not on a development, technology or innovation track. It is much desirable that the private sector, people and the government partner with each other to start the evolution of green economy in India.

According to Govindan (2012) in the last decade, India has undertaken several noticeable initiatives towards greening its growth. Through public interventions, it has prioritised such areas as water conservation, renewable energy, renewal of degraded land and solid waste recycling for energy, among other initiatives. Probably in the absence of indigenous green technology, the adopters find the imported technology costly and economically unviable. In order to enhance acceptability and adoption level of green technology, the government at various levels has been providing financial incentives through tax breaks and subsidies. Despite possible government

Table 14 Biomass power co-generation tariff in India as of March 31, 2013

State	Tariff fixed by commissions	Renewable purchase obligation (%)
Andhra Pradesh	Rs. 4.28/kWh, (2010–2011) (BM); Rs. 3.48/kWh (Cogen)	Min. 3.75 %
Chhattisgarh	Rs. 3.93/unit (2010–2011) (BM)	5 %
Gujarat	Rs. 4.40/unit (with accelerated depreciation) (BM); Rs. 4.55/unit (with accelerated depreciation) for first 10 yrs. (Cogen)	10 %
Haryana	Rs. 4.00/unit (BM); Rs.3.74/unit Cogen)	1 %
	3 % escalation (base year 2007–2008)	
Karnataka	Rs. 3.66 per unit (PPA signing date); Rs. 4.13 (10th year) (BM) at Rs.3.59/unit (PPA signing date), Rs. 4.14/unit (10th Year) (Cogen)	Min.10 %
Kerala	Rs. 2.80/unit (BM) escalated at 5 % for five years (2000–2001)	3 %
Maharashtra	Rs. 4.98 (2010–2011) (BM); Rs. 4.79/unit (Comm yr.) (Cogen)	6 %
Madhya Pradesh	Rs. 3.33 to 5.14/unit paise for 20 yrs. With escalation of 3–8 paise	0.80 %
Punjab	Rs. 5.05/unit (2010–2011) (BM); Rs. 4.57/unit (2010–2011) (Cogen) escalated at 5 % for Cogen and 5 % for BM	Min. 3 %
Rajasthan	Rs. 4.72/unit – water cooled (2010–2011); Rs. 5.17 – air cooled (2010–2011) (BM)	1.75 %
Tamil Nadu	Rs. 4.50–4.74/unit (2010–2011) (BM); Rs. 4.37–4.49/unit (2010–2011) (Cogen) (escalation 2 %)	Min. 13 %
Uttaranchal	Rs. 3.06/unit (2010–2011) – BM; Rs. 3.12/unit (2010–2011) (Cogen) (new projects)	9 %
Uttar Pradesh	Rs. 4.29/unit, for existing and Rs. 4.38 for new with escalated at 4 paise/year, base year (2006)	4 %
West Bengal	Rs. 4.36/unit fixed for 10 years – biomass	4 %
Bihar	Rs. 4.17/unit (2010–2011) – biomass	1.50 %
	Rs. 4.25/unit (2010–2011) – existing (Cogen)	
	Rs. 4.46/unit (2010–2011) – new (Cogen)	
Odisha	Rs.4.09/unit	

Source: http://www.mnre.gov.in/schemes/grid-connected/biomass-powercogen/

efforts, green growth has not gathered momentum in India. It is highly desirable that multiple level campaigns on green technology and green growth should be run for a longer duration targeting the corporate, society and individual.

India has been making visible progress in terms of making climate change and sustainable development policies. In 2008, the government unveiled the National Action Plan on Climate Change (NAPCC), which along with critical sustainable development plans encompasses eight National Missions such as the National Solar Mission, National Mission for Enhanced Energy Efficiency, National Mission on Sustainable Habitat, National Water Mission, National Mission for Sustaining the Himalayan Ecosystem, National Mission for a Green India, National Mission for Sustainable Agriculture and National Mission on Strategic Knowledge for Climate Change having independent and collective objectives to create a green economy. The above missions are going to work in all spheres of human life and environment. The NAPCC identified important strategic areas like renewable energy (solar in particular), water conservation, forest conservation, protection of natural habitat, conservation of energy, energy efficiency, technology for waste to energy, clean technology, efficient and convenient public transport system, resource efficiency and strategic knowledge centres for a green economy in India. Industry bodies like the Confederation of Indian Industry (CII) and Federation of Indian Chambers of Commerce and Industry (FICCI) have been striving hard to

Table 15 Total projected investment in the power sector in India

	Investment (million $) in reference case			Investment (million $) in (r)evolution (best case) scenario		
Technology	2011–2020	2021–2030	Investment share (%) in 2021–2030	2011–2020	2021–2030	Investment share (%) in 2021–2030
Conventional (fossil and nuclear)	179,759	261,422	60.02	92,723	14,356	1.26
Total renewables	134,113	174,110	39.98	377,116	1,124,348	98.74
Biomass	7,857	19,992	4.59	44,763	28,519	2.50
Hydro	71,946	99,718	22.90	95,184	28,391	2.49
Wind	30,853	29,349	6.74	127,929	196,461	17.25
PV	22,333	24,099	5.53	61,847	187,397	16.46
Geothermal	544	395	0.09	12,690	181,149	15.91
Solar thermal	580	389	0.09	31,920	458,107	40.23
Ocean	0	168	0.04	2,783	44,324	3.89
Total investment for 2011–2020 (under best case scenario: 469839)	313,872	435,532	100.00		1,138,704	100.00

Source: Energy [r]evolution: a sustainable India energy outlook (2012)

create a positive wave for green technology, clean energy and green economy. All possible collaborative efforts at the government, corporate and individual level should be made to build, grow and sustain green economy in India.

7.2 Investment in Renewable Energy in India

Investment in renewable technology, especially in power generation, has been growing globally. India being one of the prime renewable markets is going to attract huge investment in the future. According to *Energy* [*r*]*evolution*: *A Sustainable India Energy Outlook* (a report by Greenpeace India), in a reference case scenario about $ 174110 million will be invested in renewable power generation during 2021–2030, about a 30 % increase from 2011 to 2020. Under the best case scenario, the investment figures are expected to jump up to $ 377,116 million and $ 1,124,348 million during 2011–2020 and 2021–2030, respectively (Table 15). Even in the case of business as usual, renewable power is likely to attract about 40 % of total investment during 2021–2030. It is clearly visible that solar power and wind are the prime drivers of investment in the future. This would really transform the renewable market in India. In addition to that, the renewable heating (biomass, geothermal, solar and heat pumps) is likely to attract investment up to $ 169,347 million during 2011–2030.

7.3 Impact of Renewable Energy on Employment in India

Globally, growing renewable energy markets have been showing a clear impact on direct and indirect employment opportunities. It has been observed that renewable energy growth in the USA, China and Europe has put a considerable impact on employment. Similarly, in India such an impact is clearly visible. Renewable power generation not only provides direct employment in power plants, but a significant amount of job opportunities are created in construction and installation, manufacturing, operations and maintenance. A recent study by Greenpeace India in 2012 suggests that under reference case renewable energy is going to create more jobs compared to nonrenewable energy (power) in 2020. Under the optimistic scenario by 2020, renewable power would offer about 74 % (Table 16) of total employment in the power sector in India.

Table 16 Employment scenario in the power sector in India

			Projections (in '000) in reference case			Projections (in '000) in energy [r]evolution		
Technology	Actual jobs in 2010 ('000)	Job share (%) in 2010	2015	2020	Job share (%) in 2020	2015	2020	Job share (%) in 2020
Coal	1,142	47.5	582	467	33.97	582	467	19.35
Gas, oil and diesel	165	6.9	156	131	9.53	156	131	5.43
Nuclear	33	1.4	39	39	2.84	8	7	0.29
Total renewables	1,064.81	44.3	807.31	737.68	53.66	1,557.9	1,808.4	74.93
Biomass	825	34.3	654	566	41.17	754	654	27.10
Hydro	85	3.5	70	82	5.97	103	48	1.99
Wind	67	2.8	45	40	2.91	316	280	11.60
PV	77	3.2	29	45	3.27	210	292	12.10
Geothermal	0.9	0.0	0.5	0.3	0.02	8	34	1.41
Solar thermal	1.3	0.1	1	1.1	0.08	37	161	6.67
Ocean	0.01	0.0	0.01	0.08	0.01	3.9	24.4	1.01
Solar heat	5.5	0.2	7.5	2.6	0.19	109	292	12.10
Geothermal and heat pump	3.1	0.1	0.3	0.6	0.04	17	23	0.95
Total jobs	2,404.81	100.0	1,584.31	1,374.68	100.00	2,303.9	2,413.4	100.00

Source: Energy [r]evolution: a sustainable India energy outlook (2012)

8 Conclusion

According to the United Nation's Intergovernmental Panel on Climate Change (IPCC), the world has a total of 2,795 gigatons (Gt) worth of carbon in the form of fossil fuels and reserves. Burning just 10 % of these would take the earth over the tipping point. By 2011, humans had already emitted 531 Gt, and by 2026 probably carbon emission could exceed 1,000 Gt. A recent study conducted by the University of Hawaii predicted that Indian cities like Mumbai and Chennai will be reaching the tipping point by 2034 in the "business as usual" scenario. India is also among the top 20 countries facing the threat of a significant economic impact brought on by climate change (Goenka 2013). India's economic exposure to the impacts of extreme climate-related events could be better understood by Phailin in 2013 striking coastal Odisha and Andhra Pradesh, cloudburst in Uttarakhand (2012) and super cyclone in Odisha (1999). The recent storm Phailin caused severe damage to agriculture, power infrastructure and transportation in Odisha. According to media reports, the estimated damages could be to the tune of almost \$4.15 billion. Under such circumstances, special efforts need to be taken to protect environment, human life and nonrenewable natural resources for future generations. There is dire need for green production, green building, green energy, green transportation and moreover a green economy. No doubt developing countries like India are in rapid growth stage and require more energy to sustain high growth. However, such growth should not come at the cost of the environment and precious human life. Moreover, exhausting nonrenewable sources of energy could jeopardise the life of future generations. Efforts have been taken to promote green businesses, production, building and transportation, but much is desirable at a large scale. The government has been taking major steps to improve environmental conditions in bigger cities and industrial areas and creating alternative and sustainable models in semi-urban and rural areas. Energy conservation initiatives have been constantly taken to conserve energy at all levels and promote energy efficiency to optimally use scarce resources. International agencies have been promoting and monitoring environment-related initiatives in India. The Global Green Growth Institute suggests that emerging economies like India should work on institutionalisation, development and diffusion of green technology, capacity building and financing in terms of investment flows from various sources like government, private sector, global agencies and foreign direct investment for sustainable green growth and development.

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Green Nanotechnology: The Solution to Sustainable Development of Environment

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Abstract

The environment is undergoing constant degradation in terms of quality as well as quantity due to various developmental activities occurring for satisfaction of the growing population's needs. Nanoparticles have been existing in the environment since millions of years and also being utilized since thousands of years in many areas due to their ability to be synthesized and manipulated. Literature has shown the ability of nanoparticles for detoxification of environment with respect to their usage in wastewater treatment, dye degradation, etc. However, the conventional physical and chemical methods have also shown to affect environment as it involves use of toxic substances. Hence, the green nanotechnology has gained considerable interest in recent times as an eco-friendly alternative technology for nanotechnology products. This review highlighted the characteristics, goals, and various issues in concern, of this potential field as an ultimate solution for sustainable development of environment.

Keywords

Green chemistry • Nanoparticles • Sustainable development • Wastewater treatment

1 Introduction

The natural environment consists of physical and biological factors along with their chemical interactions that affect all living and nonliving things. It has been undergoing constant changes with growth of human civilization, which has led to the deterioration and pollution of the environment through depletion of resources like air, water, and soil, destruction of ecosystems, and extinction of wildlife (Johnson et al.

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Nanoparticles have attracted considerable attraction due to their unusual and fascinating properties over their bulk counterparts for various applications, and nanotechnology involves the engineering of functional systems at the atomic or molecular scale (Hasna et al. 2012), i.e., projected ability to construct items using techniques and tools to make complete, high-performance products. Nanotechnology has a vital role in the development of innovative methods for manufacture of new products, substitution of current equipments of production, and reformulation of new materials and chemicals possessing improved performance characteristics that would result in less consumption of energy and materials and reduce harm to the environment as well as aid in environmental remediation, thus giving possibilities to remediate problems associated with the current processes in a more sustainable manner.

Environmental applications of nanotechnology answer to questions pertaining to the development of solutions to the existing environmental issues, preventive measures for future problems resulting from the interactions of energy and materials with the environment, and possible risks, if any, posed by nanotechnology itself (Mansoori et al. 2008). The environmental impact of nanotechnology can be viewed with respect to energy applications of nanotechnology and also on the influence of nanochemistry on wastewater treatment, air purification, and energy storage devices (Zhang 2003; Hillie and Hlophe 2007; Tian et al. 2007). The broader environmental impacts of nanotechnology also need consideration which include the environmental impact of the cost, size, and availability of advanced technological devices, models to determine potential benefits of reduction or prevention of pollutants from environmental sources, potential new directions in environmental science due to advanced sensors, effect of rapid advances in health care and health management as related to the environment, impact of artificial nanoparticles in the atmosphere, and impacts for the development of nanomachines (Hutchison 2001). Early application of nanotechnology having environmental implications includes the use of zerovalent iron for remediation of soil and water contaminated with chlorinated compounds and heavy metals which proves to be a rapidly emerging technology with potential benefits. Environmental remediation involves degradation, sequestration, and other related approaches that would result in reduced risks to human and environmental receptors posed by different types of contaminants, the benefits of which would be more rapid and cost-effective cleanup of waste (Mansoori et al. 2008). Minimizing quantities and exposure to hazardous waste to air and water and provision of safe drinking water are among the prominent goals of environmental protection agencies, where nanotechnology could play a pivotal role in pollution prevention technologies (Ahmadpour et al. 2003; Shahsavand and Ahmadpour 2004; Darnault et al. 2005). Though the conventional physical and chemical methods are more popular for manufacture of nanotechnology products, they face the demerit of being environmentally toxic, and it is at this juncture where green nanotechnology gains prominence due to eco-friendliness, which incorporates goals and principles of green chemistry and green engineering.

2 Green Nanotechnology

Green nanotechnology involves the development of clean technologies to minimize potential environmental and human health risks associated with the manufacture and use of nanotechnology products and to encourage replacement of existing products with new nanoproducts that are more environmentally friendly throughout their life cycle (Schmidt 2007). It emphasizes the use of nanotechnology to uplift the environmental sustainability of processes that are currently exhibiting negative effects and primarily about making green nanoproducts and using them in support of sustainability. It has two main goals:

1. *Production of nanomaterials and products without harming the environment or human health and production of nanoproducts that*

provides solutions to environmental problems: This involves application of existing principles of green chemistry and green engineering to produce nanomaterials without employing toxic ingredients, at comparatively low temperatures and using less energy and renewable inputs wherever and whenever possible and utilizing life cycle thoughts in all stages of design and engineering. In addition, this field also means using nanotechnology to make current manufacturing processes for non-nanomaterials and more eco-friendly products. For instance, nanoscale membranes can separate desired chemical reaction products from waste materials. More efficient and less wasteful chemical reactions are possible by employment of nanoscale catalysts. Nanoscale sensors can form part of process control systems, working with nano-enabled information systems. Using alternative systems via nanotechnology is another way to "green" manufacturing processes.

2. *Development of products that benefit the environment either directly or indirectly*: Nanomaterials have been found capable of directly cleaning hazardous waste sites, desalinate water and treat pollutants. Indirectly, lightweight nanocomposites for automobiles and other means of transport have been able to save fuel and reduce materials used for production. Nanotechnology-enabled fuel cells and LEDs are capable of reducing energy from energy generation and aid in fossil fuel conservation. Self-cleaning nanoscale surfaces have the ability to reduce or eliminate many cleaning chemicals used in regular maintenance routines (Sustainable Nano Coatings 2013). Green nanotechnology has a wider view of nanomaterials and nanoproducts, ensuring minimization of unforeseen consequences and anticipation of the impacts throughout the life cycle (Klöppfer et al. 2007).

Current research involves the development of nanotechnology in solar cells which are a renewable resource (Gail 2009). The potentials of this field are already in application for provision of improved performance coatings for photovoltaic (PV) and solar thermal panels. Hydrophobic and self-cleaning properties combine to create more efficient solar panels. PV panels covered with nanotechnology coatings have found to stay cleaner for longer duration thus ensuring maintenance of maximum energy efficiency (nanoShell 2013).

3 Green Chemistry

Green chemistry, also called sustainable chemistry, is a philosophy of chemical research and engineering that encourages the design of products and processes that minimize the use and generation of hazardous substances. It differs from environmental chemistry in the fact that environmental chemistry deals with chemistry of the natural environment and of pollutant chemicals in nature. Whereas in green chemistry it seeks answers to questions regarding reduction and prevention of pollution at its source, which in turn applies to organic chemistry, inorganic chemistry, biochemistry, analytical chemistry, and physical chemistry (USEPA 2006). Three key developments have been identified in green chemistry (Noyori 2005):

1. Use of supercritical CO_2 as green solvent
2. Use of aqueous H_2O_2 for clean oxidations
3. Use of hydrogen in asymmetric synthesis

3.1 Principles of Green Chemistry

Green chemistry has 12 principles that explain what the definition means practically and covers the following concepts (Anastas and Warner 1998):

1. Design of processes to maximize the amount of raw materials that ends up as products.
2. Use of safe, environment-benign substances whenever possible.
3. Design of energy-efficient processes.
4. The best form of waste disposal: avoid creating it in the first place.

The 12 principles are as follows:

1. *Prevention*: Better to prevent waste than to treat or clean up waste after it has been created.

2. *Atom economy*: Designing of synthetic methods to maximize the incorporation of all materials used in the process into the final product.
3. *Less hazardous chemical syntheses*: Designing of synthetic methods, wherever practicable, to use and generate substances that possess little or no toxicity to human health and the environment.
4. *Designing safer chemicals*: Designing of chemical products to affect their desired function while minimizing their toxicity.
5. *Safer solvents and auxiliaries*: Use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.
6. *Design for energy efficiency*: Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.
7. *Use of renewable feedstocks*: A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.
8. *Reduce derivatives*: Unnecessary derivatization (use of blocking groups, protection/deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible because such steps require additional reagents and can generate waste.
9. *Catalysis*: Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. *Design for degradation*: Chemical products should be designed so that at the end of their function, they break down into innocuous degradation products and do not persist in the environment.
11. *Real-time analysis for pollution prevention*: Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
12. *Inherently safer chemistry for accident prevention*: Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents including releases, explosions, and fires.

Green chemistry is being increasingly used as a powerful tool for evaluation of environmental impact of nanotechnology by researchers (Schmidt 2007).

4 Green Engineering

Green engineering is the process and design of products that conserve natural resources and impact the natural environment as little as possible. The term is mostly used in connection to housing, but is also applicable to automobiles, lights, or anything that requires engineering, with incorporation of environmental principles. Green engineers are specially trained in the field with regard to making of materials in an environmentally friendly way. For example, in case of housing, they are concerned with the latest building materials and techniques, which may include the use of solar-powered devices like water heaters, solar lights or windows, and other design elements. Concepts used in automobiles that are considered environmentally friendly include hybrid technologies such as flex-fuel vehicles and electricity. The consumption of less energy could mean a chance to realize cost savings in the operations of these vehicles over time (Ken 2013).

4.1 Principles of Green Engineering

Green engineering has the following 12 principles (Anastas and Zimmerman 2003):

1. *Inherent rather than circumstantial*: Designers need to strive to ensure that all materials and energy inputs and outputs are as inherently nonhazardous as possible.
2. *Prevention instead of treatment*: Better to prevent waste than to treat after formation.
3. *Design for separation*: Designing of separation and purification operations to minimize energy consumption and material use.

4. *Maximize efficiency*: Designing of products, processes, and systems to maximize mass, energy, space, and time efficiency.
5. *Output pulled* versus *input pushed*: Products, processes, and systems should be "output pulled" rather than "input pushed" through the use of energy and materials.
6. *Conserve complexity*: Embedded entropy and complexity must be viewed as an investment when making design choices on recycle, reuse, or beneficial disposition.
7. *Durability rather than immortality*: Design goal should target durability than immortality.
8. *Meet need, minimize excess*: Design for unnecessary capacity or capability should be considered a flawed design.
9. *Minimize material diversity*: For promotion of disassembly and value retention.
10. *Integrate material and energy flows*: Design of products, processes, and systems should include integration and interconnectivity with available energy and material flows.
11. *Design for commercial "after life"*: Designing of products, processes, and systems for performance in a commercial "after life."
12. *Renewable rather than depleting*: Material and energy inputs should be renewable rather than depleting.

5 Nanobiosynthesis

Nanobiosynthesis or biogenic production of nanoparticles refers to the use of several species of bacteria, plants, yeast, and fungi for production of nanoparticles or to aid in the process. "Green" synthesis particularly refers to use of plants for production of nanoparticles. Nanobiosynthesis is of great interest due to simplicity of procedures, versatility, and environmental friendliness. Besides, the biologically fabricated nanostructures offer substantially different properties such as good adhesion, tribologically good properties, and optical and electrical properties of high interest in optoelectronics (Popescu et al. 2010). The general biosynthesis of metal nanoparticles from biological sources is depicted in Fig. 1 (Li et al. 2007b; Sharma et al. 2009; Prathna et al. 2010).

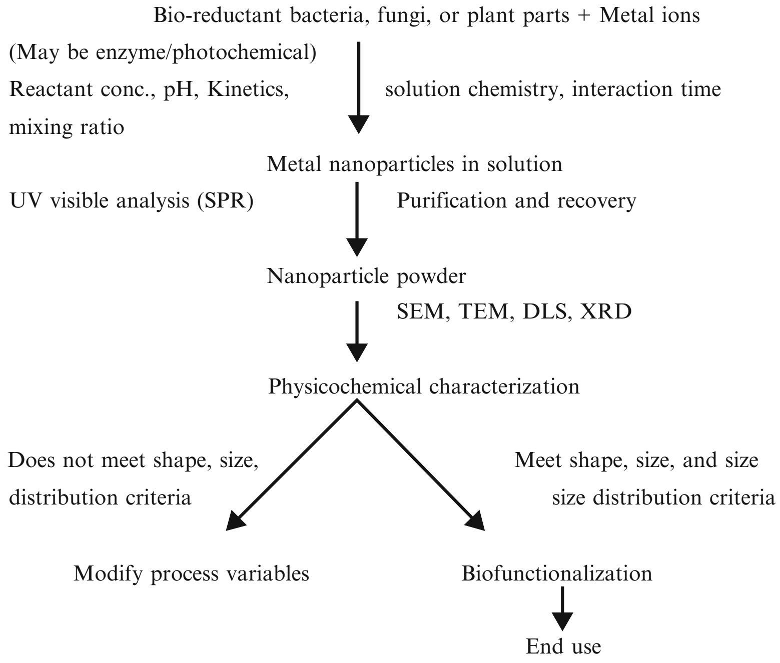

Fig. 1 Generalized flow chart for nanobiosynthesis (Prathna et al. 2010)

Nanoparticles that produced the "green" way include gold, silver, platinum, palladium, metal oxide, metal sulfide, nonmetal oxide, nanocomposites, magnetic, and alloy (Popescu et al. 2010; Song et al. 2010; Li et al. 2011; Sundrarajan and Gowri 2011; Hasna et al. 2012; Velayutham et al. 2012; Soundarrajan et al. 2012).

Microorganisms and plants have different mechanisms for nanobiosynthesis. Mechanism for nanoparticle formations varies for different microorganisms. However, they have a common path as metal ions are first trapped on the surface or inside of the microbial cells, which are then reduced to nanoparticles in the presence of enzymes. Generally, microorganisms impact mineral formation in the following two ways (Benzerara et al. 2011):

1. They modify the composition of the solution so that it becomes supersaturated or more supersaturated than it previously was with respect to a specific phase.
2. They impact mineral formation via production of organic polymers that are capable of having an impact on nucleation by favoring or inhibiting the stabilization of the very first mineral seeds.

Various mechanisms exist for nanoparticle formation by plants. Phytomining involves the use of hyperaccumulating plants to extract a metal from soil with recovery of the metal from biomass to return an economic profit (Lamb et al. 2001). Hyperaccumulator species have physiological mechanism that regulates the soil solution concentration of metals. Exudates of metal chelates from root system, for example, will allow increased flux of soluble metal complexes throughout the root membranes (Arya 2010). It has been observed that stress-tolerant plants have more capacity to reduce metal ions to the metal nanoparticles (Ankamwar et al. 2005a). Mechanism of nanobiosynthesis in plants may be associated with phytoremediation concept in plants (Huang and Cunningham 1996; Anderson et al. 1998; Haverkamp et al. 2007). Biosilicification also results in nanoparticles in cases of some higher plants as shown in Fig. 2 (Lopez et al. 2005).

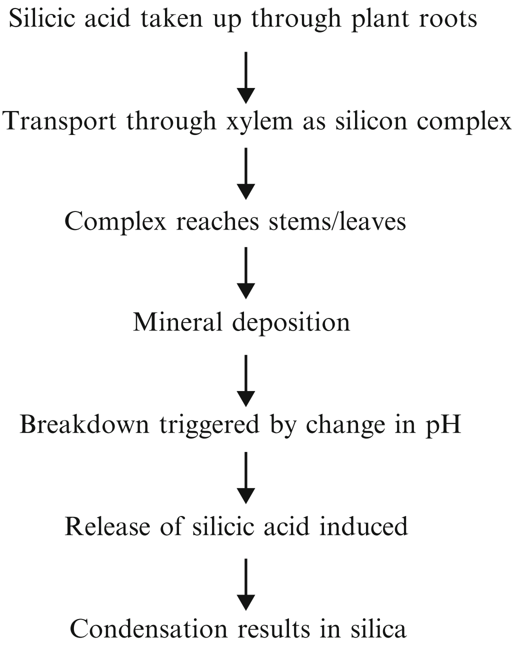

Fig. 2 Flow chart for biosilicification process

6 Role of Green Nanoparticles for Environmental Applications

Nanoparticles with antimicrobial potential like gold, silver, magnesium oxide, copper oxide, aluminum, titanium dioxide, and zinc oxide are widely used in water purification systems, in wastewater treatment, as self-cleaning and self-disinfecting agents, and as antimicrobial coatings in the wallpapers in hospitals (Ravishankar Rai and Jamuna Bai 2011). The categories of nanoparticles studied for environmental applications also include iron, bimetallics, catalytic particles, clays, carbon nanotubes, fullerenes, dendrimers, and magnetic nanoparticles (Mansoori et al. 2008).

6.1 Gold Nanoparticles

They have been precipitated within bacterial cells by incubation of cells with Au^{3+} ions (Beveridge and Murray 1980). Extracellular synthesis was reported in *Fusarium oxysporum* and *Thermomonospora* sp. and intracellular in

Verticillium sp. (Mukherjee et al. 2001, 2002; Ahmad et al. 2003a). Monodisperse particles have been synthesized using alkalotolerant *Rhodococcus* sp. under extreme biological conditions like alkaline and slightly elevated temperature conditions (Ahmad et al. 2003b). Aggregated forms of nanoparticles like gold nanotriangles have been reported in lemon grass extracts and tamarind leaf extracts (Ankamwar et al. 2005b). Extracellular synthesis of gold nanoparticles has also been observed using *Emblica officinalis* fruit extract as a reducing agent (Ankamwar et al. 2005a). Synthesis of gold nanostructures in different shapes (spherical, cubic, and octahedral) has been possible by the use of filamentous cyanobacteria from Au (I)-thiosulfate and Au (III)-chloride complexes (Lengke et al. 2006). Dead biomass of *Humulus lupulus* and leaf extract of *Ocimum basilicum* also produce gold nanoparticles (Lopez et al. 2005; Singhal et al. 2012). Gold nanoparticles are being developed for fuel cell applications which would be useful in automotive and display industry (Thompson 2007). Gold nanoparticles embedded in porous manganese oxide act as room temperature catalyst to break down volatile organic pollutants in air. Palladium-coated gold nanoparticles are very effective catalysts for removing trichloroethane (TCE) from groundwater 2,200 times better than palladium alone (Tiwari et al. 2008). The use of gold nanoparticles in colorimetric sensors enables identification of foods suitable for consumption. Other methods, such as surface-enhanced Raman spectroscopy, exploit gold nanoparticles as substrates to enable the measurement of vibrational energies of chemical bonds, which can be used for the detection of proteins, pollutants, and other label-free molecules (Ali et al. 2012).

6.2 Silver Nanoparticles

Pseudomonas stutzeri AG 259 isolated from silver mine formed silver nanoparticles when placed in silver nitrate solution (Klaus-Joerger et al. 2001). High quantity of silver nanoparticles is obtained using silver-tolerant yeast strains MKY3 (Kowshik et al. 2003). Silver nanoparticles have been reported from *Pleurotus sajor caju* along with its antimicrobial activity (Nithya and Raghunathan 2009). Extracellular biosynthesis of silver nanoparticles has been reported using marine cyanobacterium *Oscillatoria willei* NTDM01 that reduces silver ions and stabilizes the silver nanoparticles by a secreted protein (Ali et al. 2011). Silver nanoparticles have been produced in the form of a film or produced in solution or accumulated on cell surface of *Verticillium*, *Fusarium oxysporum*, or *Aspergillus flavus* (Senapati et al. 2004; Bhainsa and D'Souza 2006; Vigneshwaran et al. 2007; Jain et al. 2011). Silver nanoparticles with potential antimicrobial activity against *Escherichia coli*, *Vibrio cholerae*, *Salmonella typhimurium*, *Pseudomonas putida*, *P. vulgaris*, and *P. aeruginosa* have been reported from leaves of *Acalypha indica* and *Nicotiana tabacum*, peels of *Citrus sinensis*, and stem of *Allium cepa* (Krishnaraj et al. 2010; Saxena et al. 2010; Konwarh et al. 2011; Prasad et al. 2011). Silver nanoparticles produced with the aid of zeolite are a good sorbent for the removal of vapor-phase mercury from the flue gas of coal-fired power plants (Dong et al. 2009). They are effective antimicrobial compounds against coliform found in wastewater and incorporated as an antimicrobial, antibiotic, and antifungal agent in coatings, nanofiber, first-aid bandages, plastics, soaps, and textiles, in the treatment of certain viruses, in self-cleaning fabrics, as conductive filler, and in nanowire and certain catalyst applications (Jain and Pradeep 2005; Tiwari et al. 2008). The effect of loaded silver nanoparticles on TiO_2 has been studied for the degradation of Acid Red 88 (Anandan et al. 2008). Their presence has been found to significantly enhance DP25-TiO_2-mediated photodegradation of methyl orange at pH 6.6 (Gomathi Devi and Mohan Reddy 2010). The behavior of silver nanoparticles in a pilot wastewater treatment plant fed with municipal wastewater was investigated. TEM analyses confirmed the sorption of silver nanoparticles to wastewater biosolids, both in the sludge and effluent, and freely dispersed particles were observed only during the initial pulse spike in the effluent. XAS measurements indicated that most of the nanoparticles were present as Ag_2S in the sludge and effluent, which points to the potential of silver nanoparticles in wastewater treatment (Kaegi et al. 2011).

6.3 Palladium Nanoparticles

Palladium nanoparticles have been synthesized using coffee and tea extract at room temperature (Nadagouda and Varma 2008). Reports for its synthesis using broth of *Cinnamomum camphora* leaf are also available (Yang et al. 2010). Reaction of cyanobacterial biomass (*Plectonema boryanum* UTEX 485) with aqueous palladium (II) chloride at 250 °C for up to 28 days produced palladium nanoparticles (Lengke et al. 2007). Oleylamine-mediated synthesis of palladium nanoparticles was found useful for formic acid oxidation in $HClO_4$ solution. The catalyst showed no obvious activity degradation after 1,500 cyclic voltammetry cycles under ambient conditions, thereby holding promise as a highly active non-Pt catalyst for fuel cell applications (Mazumder and Sun 2009). Chemoselective hydrogenation of nitroarenes has been possible by the use of carbon nanofiber-supported palladium nanoparticles (Takasaki et al. 2008). Catalytically active membranes incorporated with microbially produced palladium nanoparticles have been employed for the removal of diatrizoate (Hennebel et al. 2010). Remediation of trichloroethylene has been possible by use of bioprecipitated and encapsulated palladium nanoparticles in a fixed bed reactor (Hennebel et al. 2009). Palladium nanoparticles electrodeposited on carbon ionic liquid composite electrode are useful for electrocatalytic oxidation of formaldehyde which is comparatively far superior to many of the previously reported formaldehyde sensors (Safavi et al. 2009). Photooxidation of xylenol orange is possible in the presence of palladium-modified TiO_2 catalysts, which is higher than the semiconducting support, being influenced by the size of the palladium clusters on the support (Iliev et al. 2004). Nitrogen/palladium-codoped TiO_2 enables photocatalytic degradation in 3 h for eosin yellow, which is carcinogenic and usually not easily treatable by conventional chemical or biological water treatment methods (Kuvarega et al. 2011). Palladium-modified nitrogen-doped titanium oxide showed enhanced photocatalytic degradation of humic acid over TiON within a narrow range of palladium concentration (Li et al. 2007a). Pd-modified WO_3 is an efficient tool for the decolorization of wastewater under solar light (Liu et al. 2010). New biological methods have been developed to recover precious metals from waste streams and to concomitantly produce palladium nanoparticles on bacteria, that is, bio-Pd, which serves as an effective catalyst for dehalogenation of environmental contaminants, hydrogenation, reduction, and CC reactions (Hennebel et al. 2012).

6.4 Metal Oxide Nanoparticles

Milky latex of *Calotropis procera* and *Aloe vera* extract has been used for the synthesis of “green” zinc oxide nanoparticles which are used in removal of arsenic from water (Tiwari et al. 2008; Sangeetha et al. 2011). It serves as potential UV absorbers for textiles and exhibits photocatalysis that finds application in wastewater treatment, degradation of dyes and other toxic compounds, and soil remediation (Becheri et al. 2008). Manganese-doped zinc oxide nanoparticles have been employed in the photocatalytic degradation of organic dyes (Ullah and Dutta 2008). Nanocrystalline MgO, CaO, TiO_2, and Al_2O_3 adsorb polar organics such as aldehydes and ketones in very high capacities and substantially out perform the activated carbon samples that are normally utilized for such purposes (Khaleel et al. 1999; Lucas and Klabunde 1999). Many years of research at Kansas State University, and later at Nano Scale, have clearly established the destructive adsorption capability of nanoparticles toward many hazardous substances including chlorocarbons, acid gases, common air pollutants, dimethyl methylphosphonate (DMMP), and paraoxon, 2-chloroethyl ethyl sulfide (2-CEES) and even military agents such as GD, VX, and HD (Wagner et al. 1999, 2000, 2001; Rajagopalan et al. 2002). Nanocrystalline metal oxides are particularly effective decontaminants for several classes of environmentally problematic compounds at elevated temperatures, enabling complete destruction of these compounds at considerably lower temperatures than that required for incineration (Decker et al. 2002). The application of nanocrystalline materials as destructive adsorbents for acid gases such as HCl, HBr, CO_2, H_2S, NO_X, and SO_X has been found to be more effective than commercially available oxides (Klabunde et al. 1996; Stark and Klabunde 1996; Carnes et al. 2002). In waste and wastewater treatment, MgO facilitates the adsorption and precipitation of silica and heavy metals and helps in preventing scale formation in boilers, heat exchangers, and piping. For soil remediation, it is an excellent pH modifier and heavy metal scavenger in contaminated soils and also effectively precipitates heavy metals, thus preventing subsequent leaching from treated soils (http://www.baymag.com). Copper oxide nanoparticles have been synthesized using gram-negative bacterium of the genus *Serratia* and *Aloe vera* extract (Saif Hasan et al. 2008; Sangeetha et al. 2012). Cupric oxide can safely dispose hazardous materials like cyanide, hydrocarbons, halogenated hydrocarbons, and dioxins through oxidation (Kenney and Uchida 2007). Copper oxide nanocrystals also possess photocatalytic, photovoltaic, and photoconductive functionalities (Kwak and Kim 2005). Titanium oxide has been synthesized via the “green” route using leaf extracts of *Catharanthus roseus* and *Nyctanthes arbor-tristis* and R5 peptide derived from diatom *Cylindrotheca fusiformis* and also using *Lactobacillus* sp. and Saccharomyces cerevisiae (Sewell and Wright 2006; Jha et al. 2009; Sundrarajan and Gowri 2011; Velayutham et al. 2012). It serves as photocatalyst in detoxification of wastewater (Jones et al. 2007). Semiconducting properties of TiO_2 materials are responsible for the removal of various organic pollutants (Makarova et al. 2000). Degradation of nitrobenzene has been achieved using nano-TiO_2 (Yang et al. 2007). The bacterium *Actinobacter* sp. has been shown to be capable of synthesizing iron-based nanoparticles under ambient conditions depending on the nature of precursors used (Bharde et al. 2005, 2008). They are being used to clean carbon tetrachloride in groundwater and arsenic from water wells. The use of zerovalent iron (ZVI or Fe^0) for in situ remedial treatment has been expanded to include all different kinds of contaminants (Ponder et al. 2000).

6.5 Platinum Nanoparticles

They have been synthesized using >10 % *Diospyros kaki* leaf extract as reducing agent from an aqueous $H_2PtCl_6.6H_2O$ solution at a reaction temperature of 95 °C and as reducing agent from aqueous chloroplatinic acid at a reaction temperature of 100 °C that finds application in water electrolysis (Song et al. 2010; Soundarrajan et al. 2012). Preferential oxidation of carbon monoxide is important for purification of H_2 for use in polymer electrolyte fuel cells which has been possible with platinum nanoparticles in mesoporous silica with unprecedented activity, selectivity, and durability below 353 K (Fukuoka et al. 2007).

7 Barriers and Challenges to Commercialization of Green Nanotechnology (ACS 2011)

1. Lack of clear design guidelines for researchers in initial discovery phases of green nanoscience. The choices made for the synthesis of new green nanomaterials can affect throughout the development and commercialization process which most researchers are unaware of.
2. Many green nanomaterials require new commercial production techniques, which increases the need for basic research, engineering research, and coordination of the two between the industrial and research communities, as the challenges do not appear until firms begin to produce in large quantities. This problem is common for small companies and start-ups and solutions rely at least partially on work done by the research community.

3. Lack of a “deep bench” of scientists and engineers with experience in developing green nanotechnology. The impact of this situation is mostly apparent in small and large industrial firms.
4. Need for constant development and updating of toxicology and analysis protocols to reflect advances in science. There is also a need to develop in-line process analytical and control techniques for full-scale manufacturing operations by involvement from academic researchers.
5. Regulatory uncertainty persists, and green nanotechnologies often face higher regulatory barriers than existing or conventional chemicals. This affects small and large industrial firms as they attempt to move green nanotechnologies into the market.
6. The end-market demand is unclear, especially since there are only a limited number of commercial grade products that can be compared to conventional materials in terms of performance.

8 Actions to Be Taken to Overcome the Barriers (ACS 2011)

1. Discover, uncover, and provide key analysis and characterization tools. Reduce analysis costs.
2. Develop, characterize, and test precision-engineered nanoparticles for biological and toxicological studies needed to guide greener design. Develop reference libraries that provide the relevant data required and provide them to groups that need them for testing and also hypotheses that help in redesign of materials that are greener.
3. Investigate and understand reaction mechanisms to support more efficient and precise synthesis and production techniques. Screen for barriers and develop design guidelines for commercially producible green nanomaterials.
4. Develop design guidelines for green nanomaterials for early stage researchers and material developers to support greener nanomaterial development and production.
5. Definition of green criteria for new nanomaterials for fast-track approval by the US Environmental Protection Agency that demonstrates benefits over existing materials in market and possesses no hazard.
6. Education and outreach to regulators to ensure regulatory structures for green nanotechnology reflect accurate knowledge of their intended users and potential impacts.

9 Conclusion

Green nanotechnology is indeed an eco-friendly alternative for production of nanotechnology products for sustainable development of environment by provision of solutions to tackle the ever-expanding environmental issues like environmental degradation, depletion of natural resources, water and air pollution, and aftermaths of various other kinds of pollutions. The barriers need to be overcome by the right actions. This will be possible only by constant cooperation with a team comprising experts from multiple disciplines or rather various areas including science, commerce, and statistics that come up with solutions covering wide areas and problems that need to be addressed. If the suggestions that come up from such a discussion are rightly followed and literally put into practical action, then this potential field is sure to come up as the answer to all major environmental issues and thereby the future of tomorrow in a sustainable way.

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