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Bioremediation Using Weeds

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Preface

The natural ecosystem has been negatively impacted by environmental contamination from hazardous waste products, chemical pollutants and heavy metals. These pollutants arise from both anthropogenic sources and natural sources such as hurricanes and volcanic eruptions. Bioremediation is gradually being recognized as a standard practice for the sustainable management of environment as it is more eco-friendly and low-cost remediation techniques as compared to traditional chemical and physical methods.

The International Society for Energy, Environment and Sustainability (ISEES) was founded at Indian Institute of Technology Kanpur (IIT Kanpur), India, in January 2014 with an aim to spread knowledge/awareness and catalyse research activities in the fields of energy, environment, sustainability and combustion. The society's goal is to contribute to the development of clean, affordable and secure energy resources and a sustainable environment for the society, to spread knowledge in the above-mentioned areas and to create awareness about the environmental challenges, which the world is facing today. The unique way adopted by the society was to break the conventional silos of specializations (engineering, science, environment, agriculture, biotechnology, materials, fuels, etc.) to tackle the problems related to energy, environment and sustainability in a holistic manner. This is quite evident by the participation of experts from all fields to resolve these issues. ISEES is involved in various activities such as conducting workshops, seminars and conferences in the domains of its interests. The society also recognizes the outstanding works done by the young scientists and engineers for their contributions in these fields by conferring them awards under various categories.

The Fourth International Conference on "Sustainable Energy and Environmental Challenges" (IV-SEEC) was organized under the auspices of ISEES from 27 to 29 November 2019, at NEERI, Nagpur. This conference provided a platform for discussions between eminent scientists and engineers from various countries including India, USA, China, Italy, Mexico, South Korea, Japan, Sweden, Greece, Czech Republic, Germany, Netherland and Canada. In this conference, eminent speakers from all over the world presented their views related to different aspects of energy, combustion, emissions and alternative energy resource for sustainable

development and cleaner environment. The conference presented one high-voltage plenary talk by Mrs. Rashmi Urdhwareshe, Director, Automotive Research Association of India (ARAI), Pune.

The conference included 28 technical sessions on topics related to energy and environmental sustainability including 1 plenary talk, 25 keynote talks and 54 invited talks from prominent scientists, in addition to 70+ contributed talks and 80+ poster presentations by students and researchers. The technical sessions in the conference included fuels, engine technology and emissions, coal and biomass combustion/gasification, atomization and sprays, combustion and modelling, alternative energy resources, water and water and wastewater treatment, automobile and other environmental applications, environmental challenges and sustainability, nuclear energy and other environmental challenges, clean fuels and other environmental challenges, water pollution and control, biomass and biotechnology, waste to wealth, microbiology, biotechnological and other environmental applications, waste and wastewater management, cleaner technology and environment, sustainable materials and processes, energy, environment and sustainability, technologies and approaches for clean, sensors and materials for environmental, biological processes and environmental sustainability. One of the highlights of the conference was the rapid fire poster sessions in (i) engine/fuels/emissions, (ii) environment and (III) biotechnology, where 50+ students participated with great enthusiasm and won many prizes in a fiercely competitive environment. 300+ participants and speakers attended this three days' conference, where 12 ISEES books published by Springer, Singapore, under a special dedicated series "Energy, environment and sustainability" were released. This was third time in a row that such significant and high-quality outcome has been achieved by any society in India. The conference concluded with a panel discussion on "Balancing Energy Security, Environmental Impacts and Economic Considerations: Indian Perspective", where the panellists were Dr. Anjan Ray, CSIR-IIP Dehradun; Dr. R. R. Sonde, Thermax Ltd.; Prof. Avinash Kumar Agarwal, IIT Kanpur; Dr. R. Srikanth, National Institute of Advanced Studies, Bengaluru; and Dr. Rakesh Kumar, NEERI Nagpur. The panel discussion was moderated by Prof. Ashok Pandey, Chairman, ISEES. This conference laid out the roadmap for technology development, opportunities and challenges in energy, environment and sustainability domain. All these topics are very relevant for the country and the world in the present context. We acknowledge the support received from various funding agencies and organizations for the successful conduct of the Fourth ISEES Conference IV-SEEC, where these books germinated. We would therefore like to acknowledge SERB, Government of India (special thanks to Dr. Sandeep Verma, Secretary); NEERI Nagpur (special thanks to Dr. Rakesh Kumar, Director), CSIR; and our publishing partner Springer (special thanks to Swati Meherishi).

We would like to express our sincere gratitude to large number of authors from all over the world for submitting their high-quality work in a timely manner and revising it appropriately at a short notice. We would like to express our special thanks to who reviewed various chapters of this monograph and provided their valuable suggestions to improve the manuscripts.

In this monograph, core elements of multidisciplinary bioremediation practice are addressed and environmental pollutants can be effectively remediated using weeds. Chapters include recent results and more focused on current trends of introduction to potentials of weeds in bioremediation practice. We hope that the book would be of great interest to the professionals and postgraduate students involved in weed-based bioremediation or phytoremediation and environmental research.

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Introduction

Bioremediation by Weeds

Abstract Not only weeds have adversely effect on the plant productivity, humans and animals, but also many weeds have the beneficial properties and have immense potential in environmental applications as fodder generation, bioremediation, biofuel generation, industrial, soil and water conservation resources, etc. In the field of pollution control, weeds play a main role in bioremediation, with applications that include ridding environments of biological and chemical contaminants. Not very much information is available on the use of weeds for bioremediation. Therefore, this research area needs to be discussed and expanded. In the bioremediation processes, the specific uses and mechanisms of weeds include: (1) phytoextraction or phytoaccumulation; (2) phytostabilization; (3) rhizofiltration; and (4) phytovolatilization. The bioremediation potential of the weeds can be further improved by the operation of innovative approaches in bioremediation process. Overall, this book emphasizes on weed classification, different techniques for bioremediation processes using weeds to present the broad applicability of weeds in environmental management.

Keywords Weed biomass · Pollutant removal · Bioremediation · Biofuel · Environmental management

Himalayan region is recognized as one of the biodiversity hotspots of the world where Indo-Malayan, Indo-Chinese and Indian bio-geographical realms have converged. Based on the climate and soil conditions, water availability and socio-economic aspects, different agro-ecosystems are prevalent in the Himalaya. Further, this region is inhabited by various ethnic communities and their dependence on the unique entity is high. The paucity of baseline data necessitated a compilation of the catalog of weed flora. Part I of this monograph presented weed species from north-west Himalaya, a phytogeographical distinct region. It will help

in better understanding the patterns of plant invasion but also for explicating the processes promoting weed invasion at local, regional or global scales.

An aquatic weed may be defined as an undesirable plant that needs some sort of action to reduce its undesirable impact on the economy, the environment, human health and amenity. Aquatic weeds are biologically adapted to grow in water different bodies and complete at least part of their life cycle in aqueous environments. The undesirable qualities of aquatic weeds usually dominate their good qualities. For example, aquatic weeds can clog waterways and can have severe ecological and economic consequences. They can obstruct irrigation and hydroelectric projects, create hazards for recreational boaters, impair shoreline properties, severely markedly light availability to other useful plants and cause aquatic animals to suffocate. Aquatic weeds can be controlled by several weed control methods like preventive weed control, cultural weed control, mechanical weed control, biological weed control, chemical weed control and weed control through their utilization for biofuel production. Nevertheless, aquatic weeds represent one of the most useful feedstocks that can be harnessed for biofuel production to minimize dependence on limited crude oil and natural gas. Biofuel production from weed biomass may play a crucial role in meeting growing energy demands. Certain aquatic plants, such as duckweeds, water hyacinth, water lettuce, water chestnuts and cattails, are excellent sources of biofuel production. Algae have 20–80% oil contents that could be converted into different types of biofuels such as biodiesel and biokerosene. Similarly, lignocellulosic biomass (LCB) from aquatic weeds represents a sustainable alternative feedstock for production of biofuel. Characteristics such as high dry matter yield, low nutrient/water requirements for growth and high cellulose contents make aquatic weeds very attractive as feedstock for biofuel production. The production of large-quantity weed biomass needs to be wisely managed and utilized for the production of biofuels. Gene technology may be used to enhance the production of oil and biofuel contents and stability of aquatic weeds.

Part II of this monograph focuses on the utilization of aquatic weeds as a possible substitute for fossil fuel and current research activities in bioconversion of aquatic weeds to biofuel. The effectiveness of wastewater treatment by aquatic weeds was summarized at laboratory and pilot scales. Aquatic weeds are capable of effective pollutant removal from polluted water and thus allow usage of the treated water for agricultural works. In addition, aquatic plants are also effective for eliminating several heavy metals like copper, iron and mercury (Cu^{2+}, Fe^{3+} and Hg^{2+}) from contaminated soil. Subsequently, after the wastewater treatment and metal removal, harvested biomass could be used as feedstock for the production of different biofuels. Therefore, the biomass of aquatic plants could be potential raw materials for the production of bio-ethanol, bio-hydrogen and bio-methane. The potential of several other feedstocks is also summarized in comparison with aquatic weeds for various biofuel production. In conclusion, aquatic weeds served as not only a potential biological agent for wastewater treatment and heavy metal removal from contaminated water and soil but also a potential feedstock for biofuel productions (Ali et al. 2020).

Parthenium hysterophorus L. is one of the most invasive weeds and belongs to the family Asteracaeae. The weed which is commonly called carrot grass, Santa Maria, Santa Maria fever few or famine weed is native to American tropics but has spread rapidly to over thirty countries across Africa, Asia and Australia. *Parthenium* weed not only causes huge loss to crop production and plant biodiversity owing to its aggressive dominance, but also has adverse effect on the health of humans and livestock. It is known to cause allergic respiratory problems, contact dermatitis, mutagenicity, allergic rhinitis, breathlessness and even diarrhoea in mammals, depending upon the climatic conditions. Several different approaches have been employed for management of the weed including mechanical such as cutting and burning; chemical such as chemical herbicides; and biological which includes leaf-feeding beetle, stem-galling moth, stem-boring weevil and fungi. However, each of these approaches has its own limitations, and therefore, integrated weed management (IWM) is considered the best option to effectively manage its spread (Pant et al. 2015). In spite of several disadvantages, the weed has the advantage of being able to grow profusely under wide range of climatic conditions and on marginal lands, thereby producing huge amount of biomass that can be pre-treated and used for developing value-added commercial products. Many innovative uses of this weed in the field of medicine, nano-medicine, bioremediation, commercial enzyme production and biofuel production have been proposed recently.

Seaweeds can be considered as potential industrial biotechnology feedstock to achieve the worldwide demand for energy and biochemical production. The higher production yield, growth rate and carbon dioxide fixation capacity of the seaweeds highlight them as a promising alternative to terrestrial crops. In addition, farming of macroalgae does not require arable land, freshwater or fertilizer, avoiding hostile impacts on food supplies. For maximum utilization of seaweed biomass into biorefinery, the bioprospecting of the novel seaweed degrading enzymes and pathways is necessary. Recent studies reported the alginate catabolism for the successful production of bio-ethanol in few micro-organisms including *Escherichia coli* and *Saccharomyces cerevisiae*. Efforts are required to uncover the metabolism of various polysaccharides for maximum utilization of the algal polysaccharides. Also, engineering of potent seaweed converting micro-organisms, discovery and isolation of novel polysaccharide degrading enzymes and their metabolic pathways is the new interesting area of research. Metabolic engineering and synthetic biology approaches have been developed to engineer micro-organisms capable of metabolizing unique sugars from seaweed biomass to produce biofuels (Prasad 2012).

The synthetic polymers have been a significant challenge to humans as these are often disposed in land and water where they will persist in all seasons leaking toxic chemicals in the environment. The rising environmental awareness is promoting researchers to develop new biodegradable material. Biopolymers are the form of polymers derived from biological sources or chemically synthesized using biological materials as substrates. Seaweeds are versatile organisms, able to produce diverse type of polymers, i.e. agar, agarose, carrageenan, alginate, ulvan, fucoidan and polyhydroxyalkanoates. Biopolymers derived from seaweed polysaccharides

possess promising features as they are renewable, biodegradable, biocompatible and environment-friendly. Also, seaweed has been used as reinforcement to improve the mechanical properties of polymer composites. Various modifications have been done on seaweed biopolymer to improve the properties of the materials such as blending with other polymers, the addition of compatibilizer and reinforcement with other materials. The potential of seaweed as filler in polymer composites improves the thermal, physical and mechanical properties of the synthetic polymer matrix. The seaweed-based polymers have potential applications for drug delivery, tissue engineering, regenerative medicine, wound healing management, bioremediation, food additive, food packaging, etc.

Seaweeds, the algal forms growing in the sea, have immense potential for their possible application in food, feed and various other commercially valuable products. Utilization of seaweeds for human consumption has been reported about 1700 years ago during the Neolithic period. Initially, these were harvested by coastal population mainly for food and fodder, but later on with increased demand of seaweed-derived products seaweeds find their application in several industrial processes. Seaweed farming although very simple, but at the same time it is very sensitive w.r.t. factors like reduction in water quality, reduction in coastal nutrients and other climate changes. Industrial production of seaweeds can be successfully achieved with the help of several technological interventions. However, at ground level, optimization and efficient development of new strategies are continuously desired not only for increased biomass productivity, but also for the development of quality products of commercial importance. Besides its enormous utility in industries, seaweed farming also has few very important and significant footraces that need to be dazed to explore its potential fully. A detailed framework involving all the aspects of seaweed farming, its industrial utility and socio-economic impact has been touched in order to convert the theoretical potential of seaweeds into reality.

Weeds are undesired plants that grow in a place where they are not required. Part III is the discussion about weed utilization. However, this definition is not true for some seaweed. Weed plants are generally considered noxious or harmful for humans, animals and biodiversity. They adversely affect the native plant productivity and cause health hazards and productivity loss to agriculture and agro-forestry. In the modern globalization and climate change scenario, a number of weed plants are spreading to new places and growing in a large area across the world. Therefore, the management and utilization of weed plants are of considerable importance. Weed plant biomass has immense potential for bioenergy, biomaterials and sources of important chemicals, bioactive molecules, food, feed and nutraceutical. Various unique characteristics of different weedy plants can be utilized for the benefit of society and the environment. The property of high lignocellulose content and requirements of lesser nutrients and water for the growth of weeds can be harnessed as cheap raw material resources for the production of commercially important biochemicals. Weed biomass can act as a direct source for bioactive compounds, which can be extracted using various solvent extraction

processes. Fresh biomass and residual weed biomass after biochemical extraction can also be used as raw material for microbial fermentation to produce enzymes and biochemicals such as alcohols, organic acids and bioplastic.

Invasive plants are the species that are non-native to an ecosystem and widely known as "weeds". Weeds can cause adverse economic, ecological effects, disturbance in biodiversity, extinction of indigenous plant species and the spread of human or animal diseases due to their fast growth rate, strong survival ability and fewer natural predators. Along with the pest, weeds are the main challenge to the farmers in agriculture crop production. Several methods including chemical, biological and mechanical control have been implemented for controlling the spread of weeds. However, huge weed biomass is generated globally that could create secondary pollution. Nevertheless, the weed biomass can be utilized for the production of biochar, bio-oil, syngas by pyrolysing the biomass under an oxygen-free environment. Biochar has received great attention due to varieties of applications including soil amendment to improve soil physical, chemical and biological properties, carbon sequestration, removal or immobilization of organic contaminants in soil and water. Therefore, this monograph has broadly heightened the status and potential of some terrestrial and aquatic weed plants as a source for biochemical, bioactive compounds and nutraceutical, and as a raw material for microbial fermentation to produce biochemicals and strategies to utilize the weed biomass as a feedstock for biochar preparation and its application in agriculture and environmental clean-up.

Weeds are plants that are growing where they are not desired. The weeds are responsible for various agricultural, environmental and economic losses. Weeds not only interfere with agricultural activities like crop harvesting but also are accountable for economic losses by reducing crop health, quality and yield. Weeds are also intrusive with animal feeding by causing poisoning, tainting animal products and acting as an alternative host for plant and animal pests. Besides this, they prevent the water flow by blocking the ditches and irrigation channels. Therefore, nowadays, weeds and their management are a challenging and important problem in agriculture, the environment and society. Presently, weed control is carried out by chemical methods using herbicides which are responsible for soil and water pollution. Hence, there is a need to explore alternative strategies to control invasive weeds. In recent times, biological methods have been preferred over conventional chemical methods for synthesis of nanoparticles (NPs) as they fulfil the principles of green chemistry. Plant-mediated synthesis of nanoparticles is widely used and important area of nanotechnology. The biosynthesized nanoparticles exhibited a wide range of biological activities including antibacterial, antifungal, antioxidant, larvicidal activities. Furthermore, nanoparticles were also used in textile dye degradation and wastewater remediation. Therefore, weed-based biosynthesis of nanoparticles is considered as an alternative strategy not only for the utilization of the invasive weed species but also for weed control through its harvesting and usage. The aim of this monograph provides an outline of the seaweed polysaccharide composition, different pretreatment technologies, enzyme systems for seaweed biomass conversion, native and the engineered alginate degradation

pathways in micro-organisms, application in the production of biofuels, weed-mediated phytosynthesis, characterization and applications in bioremediation.

This monograph is organized into ten different chapters. Specific topics covered in the monograph include:

- Diversity, distribution and status of weed species of north-west Himalaya
- Utilization of aqueous weeds for biofuel production: current status and future prospects
- Aquatic weeds: a potential pollutant removing agent from wastewater and polluted soil and valuable biofuel feedstock
- *Parthenium hysterophorus*: weed to value
- Seaweed biomass utilization pathways in microbes and their applications in the production of biofuels
- Seaweed-based biodegradable biopolymers, composite and blends with applications
- Seaweed cultivation and its biobusiness status around the world
- Utilization of weed plants for biochemicals and bioactive compound production
- Utilization of invasive weed biomass for biochar production and its application in agriculture and environmental clean-up
- Weed biomass-based nanoparticles and their applications.

The topics are organized into three different parts: (i) general weed diversity; (ii) bioremediation; and (iii) weed utilization.

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Anand Giri is currently working as Council of Scientific and Industrial Research (CSIR) India Research Fellow in the Department of Environmental Sciences at Central University of Himachal Pradesh, India. He pursued his M.Sc. in Environmental Science from G. B. Pant University of Agriculture and Technology, India and B.Sc. degree from Kumaun University Nainital, Uttarakhand in year of 2010. He was awarded the Junior Research Fellowship by Board of Research in Nuclear Sciences, Department of Atomic Energy in the year of 2014 and Senior Research Fellowship by CSIR, Government of India in 2018. He has extensive experience in R&D management and consultancy in the field of Environmental Impact Assessment and Management. He has published multiple research papers in national and international journals.

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Part I
General Weed Diversity

Chapter 1
Diversity, Distribution, and Status of Weed Species of Northwest Himalaya

Zishan Ahmad Wani, Shreekar Pant, and Virbala Sharma

1.1 Introduction

Weed is defined as 'a herbaceous plant not valued for use or beauty, growing wild and rank, and regarded as cumbering the ground or hindering the growth of superior vegetation' (Zimdahl 1999). Weeds are those plants which are harmful, interfere with the agricultural operations, increase labor, add input to the cultivation, and reduce the crop yield (Sen 2000). Weeds grow in a variety of ecosystems including pastures, rangelands, and forests. There are approximately 250,000 species of plants worldwide, of those about 3% or 8000 species act as weeds (Kumari 2016). Weeds have been recognized as a problem since the beginning of agriculture and the battle against weeds is a never ending one (Tiwari et al. 2016). Weeds are believed to have been existing on the earth ever since the man started cultivating plants around 10,000 BC (Macneish 1964). The factors that create weed problems can be classified as: hydrological, habitat modification, changes in succession, disturbances, grazing, competition, diseases, hybridization, reproductive constraints, introduction, etc. (Reid 1998). Weeds differ from other plants in being more adaptive and having peculiar characteristics that make them more competitive (Dangwal et al. 2010). Most of the weeds have characteristics of enormous seed production, variety of seed dormancies, ability to grow and multiply under variable environmental conditions (Sharma et al. 2010). Some plants are naturally weedy and become a nuisance when agriculture invades the areas in which they already grow, whilst others have developed into weeds since people have started to cultivate crops

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(Swarbrick and Mercado 1987). Weeds reduce crop yield by competing for water, soil moisture, soil nutrients, sunlight, and growing space needed by crop plants. Weeds have the ability to spread rapidly and reproduce in high numbers which enables them to effectively crowd out native and endemic plant populations and establish a plant kingdom of their own within a short period of time. Weeds also act as alternate hosts for insects, bacteria, viruses, and nematodes that affect the crops badly by causing diseases (Younkin 1942). Weeds also inhibit the growth parameters of crop plants by secreting allelopathic chemicals (Oudhia and Tripathi 1998). Most of the weeds are exotic and have been introduced for various purposes like food, fodder, medicinal, ornamental, plantation, horticulture, etc., and they support farming and forestry in a big way. Introduced species became invasive when they are introduced deliberately or unintentionally outside their natural habitats into new areas where they express the capability to establish, invade, and out-compete native species (Sekar et al. 2012). These invasive species are threatening biodiversity by exerting significant impact on the native and endemic plant species or directly by altering ecosystem properties and resulting in the displacement of native communities, hence creating an imbalance in natural and agricultural ecosystems (Vitousek 1986; Kohli et al. 2004). Several exotic disturbances dramatically affect succession and lead to exotic annual communities with low native species richness (Stylinski and Allen 1999).

Most often, the term 'weed' is used to denote the invasive species only, but native plants have also the weed potential and can also compete with our crops. Invasive species are defined as the exotic/non-native plants that express the capability to invade or compete with native species. But a weed may be a native or an exotic species that mostly grow and compete with our crops, most of which are non-native. Thus, weeds are both native and non-native, whereas 'invasive' is a term related to non-native competing species only. *Artemisia roxburghiana* is regarded as a weed in Indian Himalayan Region (IHR) (Bisht 2017), but it is native to the Himalayan Region (Samant et al. 1998), so cannot be regarded as invasive as it is a native plant species. The two terms 'weeds' and 'invasive species' are often used together and are considered one and the same thing. Invasive species can be regarded as weeds, but weeds cannot be regarded as invasive. The present study presents a comprehensive database of native as well as non-native weeds of Northwest Himalayan Region (Himachal Pradesh, Uttarakhand, and Jammu and Kashmir) along with common names, life forms, range of altitude, nativity, and flowering periods and will provide the baseline information about the weeds which will serve as a manual for future weed identification.

Present findings are based on the intensive review of available information on weed species for the Northwest and West Himalaya and also the survey conducted in different parts of Uttarakhand, Himachal Pradesh, and Jammu and Kashmir. Information on various aspects like altitude, life form, flowering period, and nativity was gathered. The samples of the specimens were collected and identified with the help of local/regional floras and research papers (Osmaston 1927; Choudhery and Wadhwa 1984; Naithani 1984; Samant 1987; Singh et al. 2002). Date were compiled and analyzed for diversity, distribution pattern, and nativity.

Nativity of the species was identified following Anonymous (1883–1970), Samant et al. (1998), Samant and Pal (2003), and Dhar et al. (2002).

1.2 Species Diversity and Distribution Pattern

A total of three hundred twenty-three weeds belong to 221 genera and 72 families (Table 1.1); they are of 279 species herbs, 27 shrubs, 15 climbers, and 2 species ferns (Fig. 1.1). Among the families, Asteraceae was the dominant family (57 species) followed by Poaceae (21 species), Lamiaceae (20 species), Ranunculaceae (19 species), Fabaceae (15 species), Brassicaceae (14 species) representing maximum weed species (Fig. 1.2). Out of documented weed species, 278 species represented dicot species within 186 genera and 58 families, while 43 species represented monocots within 33 genera and 12 families and 2 species as pteridophytes within 2 genera and 2 families.

Of the total species, maximum number of species (276) occur in the altitudinal zone, 1601–2400 m, followed by (206) in zone 801–1600 m, (146) in 2101–3200, (96) in up to 800 m, (54 species) in altitudinal zone 3201–4000 m, (18 species) in 4001–4800 (18), and (04 species) above 4800 m, respectively (Fig. 1.3).

Ranunculus was the dominant genera (09 species) followed by *Veronica, Ipomoea, and Artemisia* (05 species each), and *Polygonum, Chenopodium,* and *Viola* (04 species, each).

1.2.1 Richness of Native and Non-native Species

Out of 323 plant species, 56 were native to Himalayan Region and 267 plant species were non-native or exotic (Fig. 1.4). The native species grows within an altitude range of 1000–5600 m, whereas the non-native species grows within an altitudinal range of 200–4500. Only few non-native species grows above an altitude of 4000 m. Further, the peak flowering period of the recorded weeds is between May and September.

Weeds are the undesirable plants that reduce the crop yield by competing with the crops for moisture, light, space, nutrients, etc. Weeds affect everyone in the world by reducing crop yield and quality, delaying or interfering with harvesting, interfering with animal feeding, etc. (Kraehmer and Baur 2013). There is no reliable study of worldwide damage due to weeds. However, it is estimated that loss caused by weeds has exceeded the loss from any other category of agricultural pests such as insects, nematodes, diseases, and rodents (Abouziena and Haggag 2016). Generally, weeds are invasive in nature but some weeds are native too. Invasive species cause loss of biodiversity including species extinction, changes in hydrology, and ecosystem function (Sekar 2012). Besides non-native weed species, native species also act as weeds and may cause damage to the crops or may reduce the

Table 1.1 Diversity, distribution pattern of weed plants of Northwest and West Himalayan Region

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
1	*Barleria cristata* L.	Acanthaceae	Bluebell Barleria	S	200–2000	Asia	March–Sept	Bisht (2017)
2	*Dicliptera bupleuroides* Nees	Acanthaceae	Roxburgh's Foldwing	H	500–2000	Asia	March–Aug	Bisht (2017)
3	*Dicliptera roxburghiana* Nees	Acanthaceae	Chinese Foldwing	H	1500–2100	Asia	July–Oct	John and Dube (1995)
4	*Justicia parviflora* Nees	Acanthaceae	Small Flowered Rungia	H	300–2000	Asia	Oct–Dec	Bisht (2017)
5	*Peristrophe speciosa* (Roxb.) Nees	Acanthaceae	Showy Foldwing	H	Up to 1600	Asia	June–Oct	Bisht (2017)
6	*Adiantum capillus-veneris* L.	Adiantaceae	Maidenhair Fern	Pt	Up to 1800	Eurasia	_	Banday et al. (2017)
7	*Sagittaria sagittifolia* L.	Alismataceae	Arrowhead	H	Up to 1800	North America Europe	July–Aug	Ganie et al. (2015)
8	*Achyranthes aspera* L.	Amaranthaceae	Chaf Flower	H	Up to 1700	North America	July–Sept	Tiwari et al. (2016)
9	*Achyranthes bidentata* Bl.	Amaranthaceae	Ox Knee	H	1500–2200	Asia	July–Sept	Bisht (2017)
10	*Aerva lanata* (L.) Juss. ex Schult	Amaranthaceae	Mountain Knotweed	H	Up to 1200	Asia and Africa	Aug–Oct	Rawat and Kharwal (2014)
11	*Alternanthera philoxeroides* (Mart.) Griseb	Amaranthaceae	Alligator Weed	H	Up to 1900	South America	July–Nov	Masoodi and Khan (2012)
12	*Alternanthera pungens* Kunth	Amaranthaceae	Khaki Weed	H	Up to 1800	South America	May–Aug	Singh and Dangwal (2014)
13	*Alternanthera sessilis* (L.) DC.	Amaranthaceae	Joy Wood	H	Up to 1500	South America	July–Sept	Dangwal et al. (2012)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
14	*Amaranthus spinosus* L.	Amaranthaceae	Spiny Amaranth	H	1600–2800	South America	July–August	Banday et al. (2017)
15	*Amaranthus tricolor* L.	Amaranthaceae	Edible Amaranth	H	1200–1900	Asia and Africa	Aug–Nov	Dangwal et al. (2012)
16	*Amaranthus viridis* L.	Amaranthaceae	Slender Pigweed	H	Up to 2000	North America	July–Sept	Tiwari et al. (2016)
17	*Digera muricata* (L.) Mart.	Amaranthaceae	False Amaranth	H	500–1200	Asia and Africa	Aug–Sept	Dangwal et al. (2012)
18	*Narcissus pseudonarcissus* L.	Amaryllidaceae	Wild Daffodil	H	1600–2200	Europe	March–April	Banday et al. (2017)
19	*Bupleurum hamiltonii* Bal.	Apiaceae		H	2200–4500	Asia	July–Sept	Bisht (2017)
20	*Bupleurum lanceolatum* Wall. ex DC.	Apiaceae	Lanceleaf Thorough-Wax	H	1600–2600	Asia and Africa	July–Sept	Kumar et al. (2018)
21	*Centella asiatica* (L.) Urb.	Apiaceae	Pennywort	H	Up to 2100	Asia	April–Nov	Bisht (2017)
22	*Conium maculatum* L.	Apiaceae	Hemlock	H	1500–2400	Europe	June–Aug	Kumar et al. (2018)
23	*Heracleum candicans* Wall. ex DC.	Apiaceae	White Leaf Hogweed	H	2000–4500	Himalayan Region	June–Aug	Tiwari et al. (2016)
24	*Pimpinella diversifolia DC.*	Apiaceae		H	2000–3200	Himalayan Region	June–Sept	Bisht (2017)
25	*Scandix pectin-veneris* L.	Apiaceae	Venus Comb	H	1600–3100	Eurasia	May–Sept	Rawat and Kharwal (2014)
26	*Arisaema concinnum* Sch.	Araceae	Chinese Cobra	H	1500–2200	Himalayan Region	May–July	Kumar et al. (2018)
27	*Hedera helix* (L.) Pran.	Araliaceae	Common Ivy	C	1600–2600	Europe	Sept–Nov	Tiwari et al. (2016)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
28	*Hedera nepalensis* K. Koch	Araliaceae	Himalayan Ivy	C	1000–3000	Asia	Sept–Nov	Tiwari et al. (2016)
29	*Asparagus racemosus* Willd	Asparagaceae	Buttermilk Root	S	1000–2000	Asia and Africa	July–Aug	Banday et al. (2017)
30	*Asphodelus tenuifolius* Cav.	Asphodelaceae	Narrow Leaves Asphodel	H	Up to 1700	Asia	April–June	Banday et al. (2017)
31	*Achillea millefolium* L.	Asteraceae	Yarrow	H	1800–3100	Europe	June–July	Ganie et al. (2016)
32	*Ageratum conyzoides* L.	Asteraceae	Goat Weed	H	200–2000	South America	June–Aug	Kumar et al. (2018)
33	*Ageratum houstonianum* Miller	Asteraceae	Floss Flower	H	200–1500	North America	May–Oct	Tiwari et al. (2016)
34	*Anaphalis adnata* Wall. ex DC.	Asteraceae	Pearly Everlasting	H	1500–2600	Asia	May–Oct	Bisht (2017)
35	*Anaphalis contorta* (D. Don) Hook.	Asteraceae	Eared Leaf Pearly Everlasting	H	1500–4500	Himalayan Region	June–Oct	Bisht (2017)*
36	*Anaphalis triplinervis* (Sims) C.B. Clarke	Asteraceae	Triple-Veined Pearly	H	1500–3800	Himalayan Region	June–Sept	Tiwari et al. (2016)
37	*Anthemis cotula* L.	Asteraceae	Stinking Chamomile	H	1600–2500	Europe	May–Sept	Banday et al. (2017)
38	*Arctium lappa* L.	Asteraceae	Burdock	H	1600–3200	Europe	June–August	Banday et al. (2017)
39	*Artemisia annua* L.	Asteraceae	Annual Wormwood	H	1500–2400	Asia	July–Oct	Tiwari et al. (2016)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
40	*Artemisia capillaris* Thunb.	Asteraceae	–	H	1500–4300	Asia	June–Sept	Dobhal et al. (2006)
41	*Artemisia nilagirica* (Clarke) Pamp	Asteraceae	Indian Wormwood	S	Up to 2400	Asia	July–Nov	Tiwari et al. (2016)
42	*Artemisia roxburghiana* Wall. ex Besser	Asteraceae	Roxburgh's Wormwood	H	2200–5600	Himalayan Region	June–Sept	Bisht (2017)
43	*Artemisia tournefortiana* Reichb.	Asteraceae	–	H	1700–2100	Asia	June–July	Banday et al. (2017)
44	*Aster peduncularis* Wall. ex Nees.	Asteraceae	Himalayan Aster	H	1800–3200	Himalayan Region	June–Sept	Bisht (2017)
45	*Bellis perennis* L.	Asteraceae	Common Daisy	H	1600–2100	Europe	June–Sept	Tiwari et al. (2016)
46	*Bidens bipinnata* L.	Asteraceae	Spanish Needle	H	1500–2200	South America	June–Aug	Tiwari et al. (2016)
47	*Bidens pilosa* L.	Asteraceae	Beggar Tick	H	1900–2700	South America	March–Aug	Dangwal et al. (2012)
48	*Caesulia axillaris* Roxb.	Asteraceae	Pink Node Flower	H	200–1500	Asia	June–Aug	Kabdal et al. (2014)
49	*Carpesium abrotanoides* L.	Asteraceae	Pig's Head	H	1000–2200	Eurasia	Sept–Nov	Bahaar and Bhat (2012)
50	*Centaurea iberica* Trev.ex Spreng.	Asteraceae	Iberian Star Thistle	H	2500–3500	Eurasia	July–August	Banday et al. (2017)
51	*Centipeda minima* (L.) Braon and Ascherson	Asteraceae	Sneeze Wort	H	1200–2200	Australia Asia	July–Sept	John and Dube (1995)
52	*Cichorium intybus* L.	Asteraceae	Chicory	H	1600–2500	Europe	March–Sept	Banday et al. (2017)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
53	*Chromolaena adenophorum* L.	Asteraceae	Crofton Weed	H	500–2000	North America	July–Sept	Angiras (2014)
54	*Circium vulgare* (Savi.) Tenore	Asteraceae	Bull Thistle	H	1500–2200	Eurasia	July–Aug	Tiwari et al. (2016)
55	*Cirsium arvense* (L.) Scop.	Asteraceae	Creeping Thistle	H	1600–2700	Eurasia	June–Oct	Singh and Dangwal (2014)
56	*Conyza bonariensis* (L.) Cronq.	Asteraceae	Asthma Weed	H	1500–2800	North America	April–Sept	Tiwari et al. (2016)
57	*Conyza canadensis* (L.) Cronq.	Asteraceae	Horseweed	H	1600–2500	South America	June–Sept	Banday et al. (2017)
58	*Cousinia microcarpa* Boiss.	Asteraceae	–	H	2500–4600	Asia	July–Sept	Kumar et al. (2018)
59	*Eclipta alba* (L.) Hassk.	Asteraceae	False Daisy	H	1200–2000	North America–Asia	Jan–Dec	Kumar et al. (2018)
60	*Elephantopus scaber* L.	Asteraceae	Elephant Foot	H	Up to 1800	Asia Africa	Sept–Nov	Bisht (2017)
61	*Emilia sonchifolia* (L.) DC.	Asteraceae	Lilac Tassel Flower	H	Up to 1500	Asia and Africa	July–Oct	Bisht (2017)
62	*Eupatorium adenophorum* Spreng.	Asteraceae	Croton Weed	H	Up 2000	North America	Feb–Aug	Negi (2016)
63	*Galinsoga ciliata* (Raf.) Blake	Asteraceae	Small Flowered Galinsoga	H	1500–2200	South America	July–Sept	Dangwal et al. (2012)
64	*Galinsoga parviflora* Cav.	Asteraceae	Quick Weed	H	1000–3000	South America	June–Sept	Dangwal et al. (2012)
65	*Gerbera gossypina* (Royle) Beauv.	Asteraceae	Hairy Gerbera Daisy	H	1500–2200	Himalayan Region	May–Aug	Bisht (2017)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
66	*Gnaphalium affine* D. Don.	Asteraceae	Croton Weed	H	1700–2500	Africa Europe	Mar–June	Bahaar and Bhat (2012)
67	*Gnaphalium hypoleucum* DC.	Asteraceae	–	H	1500–2400	Asia	May–Aug	Bisht (2017)
68	*Gnaphalium luteo-album* L.	Asteraceae	Bal Raksha	H	1500–2000	Europe	Jan–Dec	Gupta et al. (2008)
69	*Inula cappa* DC.	Asteraceae	Fragrant Inula	H	1500–2200	Himalayan Region	June–Sept	Bisht (2017)
70	*Inula cupsidata* Wall. ex DC.	Asteraceae	Lanceleaf Inula	S	1600–2200	Himalayan Region	July–Sept	Bisht (2017)
71	*Lactuca serriola* L.	Asteraceae	Prickly Lettuce	H	1200–1800	Eurasia–Africa	July–Sept	Banday et al. (2017)
72	*Myriactis nepalensis* Less.	Asteraceae	Nepal Myriactis	H	1500–3300	Himalayan Region	April–Nov	Kumar et al. (2018)
73	*Parthenium hysterophorus* L.	Asteraceae	Santa Maria Feverfew	H	500–1800	South America	Jan–Dec	Dangwal et al. (2012)
74	*Saussurea heteromalla* D. Don	Asteraceae	–	H	2500–3800	Eurasia	April–August	Bisht (2017)
75	*Senecio vulgaris* L.	Asteraceae	Common Groundsel	H	1500–2000	Europe	April–May	Banday et al. (2017)
76	*Siegesbeckia orientalis* L.	Asteraceae	St. Paul's Wort	H	400–2400	Africa	August–Sept	Bisht (2017)
77	*Silybum marianum* (L.) Gaertn.	Asteraceae	Milk Thistle	H	1500–2000	Africa–Europe	June–Aug	Singh and Dangwal (2014)
78	*Solidago canadensis* L.	Asteraceae	Canada Golden Rod	H	1500–2200	North America	Oct–Dec	Dangwal et al (2011)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
79	*Solidago virgaurea* L.	Asteraceae	European Golden Rod	H	1500–2200	Eurasia–Africa	Sept–Dec	Bisht (2017)
80	*Sonchus asper* (L.) Hill	Asteraceae	Prickly Sowthistle	H	800–2000	Eurasia	May–Sept	Banday et al. (2017)
81	*Sonchus oleraceus* L.	Asteraceae	Common Sowthistle	H	1500–2500	Cosmopolitan	March–Nov	Kumar et al. (2018)
82	*Tagetes minuta* L.	Asteraceae	Wild Marigold	H	1000–2600	South America	August–Oct	Tiwari et al. (2016)
83	*Taraxacum officinale* Wigg.	Asteraceae	Dandelion	H	1500–4000	Europe	April–Sept	Banday et al. (2017)
84	*Tragopogon graciles* D. Don	Asteraceae	Slender Salsify	H	1500–3200	Himalayan Region	March–Sept	Dangwal et al. (2012)
85	*Tridax procumbens* L.	Asteraceae	Tridax Daisy	H	Up to 1500	South America	June–Sept	Singh and Dangwal (2014)
86	*Xanthium spinosum* L.	Asteraceae	Spiny Cocklebur	H	1600–2200	South America	July–Oct	Banday et al. (2017)
87	*Xanthium strumarium* L.	Asteraceae	Rough Cocklebur	S	1200–2500	Africa–South America	Aug–Sept	Banday et al (2017)
88	*Impatiens glandulifera* Royle	Balsaminaceae	Himalayan Balsam	H	2200–3800	Himalayan Region	June–Oct	Bahaar and Bhat (2012)
89	*Begonia picta* Smith	Begoniaceae	Begonia	H	1000–2800	Himalayan Region	Aug–Oct	Bisht (2017)
90	*Berberis aristata* Roxb.	Berberidaceae	Indian Barberry	S	1500–3000	Himalayan Region	June–Nov	Tiwari et al. (2016)
91	*Berberis lycium* Royle	Berberidaceae	Barberry	S	1700–2600	Himalayan Region	March–June	Kumar et al. (2018)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
92	*Cynoglossum denticulatum* DC.	Boraginaceae	Barbed Forget Me Not	H	1500–2200	Asia and Africa	June–Sept	Kumar et al. (2018)
93	*Myosotis palustris* (L.) Nath.	Boraginaceae	True Forget Me Not	H	1500–2000	North America	May–June	Banday et al. (2017)
94	*Arabis amplexicaulis* Edgew.	Brassicaceae	Stem-Clasping Rock-Cress	H	1800–3200	Asia	May–July	Tiwari et al. (2016)
95	*Arabis glabra* (L.) Bern.	Brassicaceae	Tower Mustard	H	1600–2800	Eurasia–North America	May–July	Melkhania and Singh (1983)
96	*Capsella bursa pastoris* L.	Brassicaceae	Shepherd's Purse	H	1500–3500	Asia and Africa	April–Sept	Banday et al. (2017)
97	*Cardamine impatiens* L.	Brassicaceae	Narrow Leaf Bitter Cress	H	2700–3500	Asia	May–June	Bisht (2017)
98	*Coronopus didymus* (L.) Smith	Brassicaceae	–	H	1500–3200	North America	March–Oct	Kumari and Saini (2018)
99	*Descurainia sophia* (L.) Webb. & Berth.	Brassicaceae	Fixweed	H	1700–2900	Africa	April–June	Banday et al. (2017)
100	*Eruca sativa* Mill.	Brassicaceae	Rocket	H	1700–2200	Europe	Jan–Dec	Banday et al. (2017)
101	*Goldbachia laevigata* (M. Bieb.) DC.	Brassicaceae	–	H	Up to 1400	Asia	June–Sept	Kumar et al. (2018)
102	*Lepidium capitatum* Hk. T.	Brassicaceae	Himalayan Peppergrass	H	3500–5300	Himalayan Region	July–Sept	Bahaar and Bhat (2012)
103	*Nasturtium officinale* Ait.	Brassicaceae	Water Cress	H	1600–2100	Europe	May–Aug	Dangwal et al. (2012)
104	*Rorippa indica* (L.) Hiern.	Brassicaceae	Indian Field Cress	H	Up to 1500	Asia	June–Aug	Bisht (2017)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
105	*Rorippa islandica* (Oeder) Borbas	Brassicaceae	Marsh Cress	H	1200–1800	Eurasia	June–Aug	Banday et al. (2017)
106	*Sisymbrium loeselli* L.	Brassicaceae	False London Rocket	H	1600–2500	Europe–Africa	June–Sept	Banday et al. (2017)
107	*Thlaspi arvense* L.	Brassicaceae	Penny Cress	H	2000–4500	Europe	July–Sept	Bisht (2017)
108	*Campanula colorata* (Wall.) Roxb.	Campanulaceae	Bellflower	H	1500–2000	Asia	May–June	Melkhania and Singh (1983)
109	*Cannabis sativa* L.	Cannbinaceae	Hemp	H	800–3000	Asia	July–Sept	Banday et al. (2017)
110	*Cerastium viscosum* L.	Caryophyllaceae	Clammy Chickweed	H	2000–4000	Eurasia	May–Aug	Banday et al. (2017)
111	*Drymaria cordata* (L.) *Willd*	Caryophyllaceae	Tropical Chickweed	H	200–2400	Africa	May–Sept	Bisht (2017)
112	*Gypsophila cerastioides* D. Don	Caryophyllaceae	Himalayan Baby's Breath	H	3000–4600	Himalayan Region	May–July	Bisht (2017)
113	*Silene conoidea* L.	Caryophyllaceae	Cone Champion	H	1800–3100	Asia	Mar–May	Rawat and Kharwal (2014)
114	*Stellaria media* (L.) Vill	Caryophyllaceae	Common Chickweed	H	1600–2900	Europe	June–August	Banday et al. (2017)
115	*Chenopodium album* L.	Chenopodiaceae	Common Lambs Quarter	H	1600–2900	Europe	June–Aug	Tiwari et al. (2016)
116	*Chenopodium ambrosoides* L.	Chenopodiaceae	Mexican Chai	H	1700–2200	South America	June–Aug	Dangwal et al. (2012)
117	*Chenopodium botrys* L.	Chenopodiaceae	Sticky Goosefoot	H	1600–3700	Africa–Europe	July–Sept	John and Dube (1995)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
118	*Chenopodium murale* (L.)	Chenopodiaceae	Nettle-Leaved Goosefoot	H	1500–2000	Africa–Europe	June–Aug	Dangwal et al. (2012)
119	*Cleome viscosa* L.	Cleomaceae	Asian Spider Flower	H	1200–1800	Asia	July–Oct	Dangwal et al. (2012)
120	*Colchicum luteum* Baker	Colchicaceae	Yellow Saffron	H	1900–2700	Himalayan Region	March–May	Banday et al. (2017)
121	*Commelina benghalensis* L.	Commelinaceae	Wandering Jaw	H	Up to 1600	Asia and Africa	April–Aug	Tiwari et al. (2016)
122	*Commelina diffusa* Burm.	Commelinaceae	Climbing Dayflower	H	Up to 1700	Asia	May–Nov	Tiwari et al. (2016)
123	*Commelina forskaolii* Vahl	Commelinaceae	Rat's Ear	H	1600–2800	Asia, RICA	May–Sept	Kumar et al. (2018)
124	*Cyanotis vaga* (Lour.) Sch.	Commelinaceae	Wandering Dew Grass	H	800–2500	Asia	July–Sept	Dangwal et al. (2012)
125	*Cynotis cristata* (L.) D. Don	Commelinaceae	–	H	Up to 1700	Asia and Africa	Aug–Oct	John and Dube (1995)
126	*Murdannia spirata* (L.) Bruckn.	Commelinaceae	Asiatic Dewflower	H	500–1800	Asia	Sept–Nov	John and Dube (1995)
127	*Convolvulus arvensis* L.	Convolvulaceae	Field Bindweed	C	2000–4000	Europe	April–Sept	Kumar et al. (2018)
128	*Ipomoea cairica* (L.) Sweet	Convolvulaceae	Mile-a-Minute Vine	C	700–2000	Africa–South America	Aug–Nov	Tiwari et al. (2016)
129	*Ipomoea eriocarpa* R. Br.	Convolvulaceae	Tiny Morning Glory	C	1000–1900	Asia–Australia	Sept–Nov	Dangwal et al. (2012)
130	*Ipomoea nil* (L.) Roth.	Convolvulaceae	–	C	Up to 1700	North America	March–Dec	Dangwal et al. (2012)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
131	*Ipomoea pes-tigridis* L.	Convolvulaceae	Morning Glory	C	Up to 1600	Africa	Aug–Nov	Dangwal and Singh (2012)
132	*Ipomoea purpurea* (L.) Roth.	Convolvulaceae	Common Morning Glory	C	1500–2100	South America	July–August	Dangwal et al. (2012)
133	*Diplocyclos palmatus* (L.) Jeff.	Cucurbitaceae	Striped Cucumber	C	1000–2200	Africa	June–July	Tiwari et al. (2016)
134	*Trichosanthes cucumerina* L.	Cucurbitaceae	Wild Snake Gourd	C	500–1600	Asia–Australia	June–Aug	Dangwal et al. (2011)
135	*Cyperus difformis* L.	Cyperaceae	Small Flower Umbrella Sedge	H	1600–3100	Africa–Europe	June–Sept	Tiwari et al. (2016)
136	*Cyperus iria* L.	Cyperaceae	Umbrella Sedge	H	1600–2100	Asia and Africa	July–Sept	Dangwal et al. (2012)
137	*Cyperus paniceus* (Rott.) Boeck	Cyperaceae	–	H	1500–2200	Australia	June–Sept	Dangwal et al. (2012)
138	*Cyperus sanguinolentus* Vahl	Cyperaceae	Purple Glume Flat Sedge	H	1200–1900	South America	July–Sept	Dangwal et al. (2012)
139	*Eriophorum comosum* (Wall.) Nees	Cyperaceae	Hairy Cotton Grass	H	500–2800	Asia	May–Nov	Singh and Dangwal (2014)
140	*Isolepsis setacea* (L.) R.Br.	Cyperaceae	Bristle Club Rush	H	1600–2500	Eurasia–Africa	July–Aug	Dangwal et al. (2012)
141	*Euphorbia heliscopia* L.	Euphorbiaceae	Umbrella Milkweed	H	1600–2500	Eurasia	July–Sept	Banday et al. (2017)
142	*Euphorbia hirta* L.	Euphorbiaceae	–	H	Up to 2000	North America	Sept–Oct	Kumar et al. (2018)
143	*Alysicarpus vaginalis* (L.) DC.	Fabaceae	Buffalo Clover	H	500–1500	Asia and Africa	July–Sept	John and Dube (1995)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
144	*Astragalus leucocephalus* Grah. ex Benth.	Fabaceae	White Head Milkvetch	S	1500–4700	Asia	May–Aug	Bisht (2017)
145	*Crotolaria albida* Heyne ex Roth.	Fabaceae	Narrow Leaf Rattlepod	H	500–2800	Asia	July–Nov	Bisht (2017)
146	*Crotolaria medicaginea* Lamk.	Fabaceae	Medick Rattlepod	H	200–1400	Asia	March–Aug	Dangwal et al. (2011)
147	*Lathyrus aphaca* L.	Fabaceae	Yellow Pea	H	Up to 1600	Europe	Apr–May	Rawat and Kharwal (2014)
148	*Lathyrus sativus* L.	Fabaceae	Chicken Pea	H	Up to 2500	Europe	May–Aug	Rawat and Kharwal (2014)
149	*Lathyrus sphaericus* Retz.	Fabaceae	Red Grass Pea	H	Up to 1800	Europe–Australia	May–Aug	Gupta et al. (2008)
150	*Lotus corniculatus* L.	Fabaceae	Bird's Foot Trefoil	H	1600–2500	Eurasia	May–Aug	Banday et al. (2017)
151	*Medicago sativa* L.	Fabaceae	Alfalfa	H	1600–1900	Africa–Europe	June–August	Banday et al. (2017)
152	*Melilotus indica* (L.) Allioni	Fabaceae	Yellow Sweet Clover	H	1500–2200	Eurasia–Africa	August–Sept	Tiwari et al. (2016)
153	*Trifolium pratense* L.	Fabaceae	Red Clover	H	1600–3300	Eurasia	May–August	Tiwari et al. (2016)
154	*Trifolium repens* L.	Fabaceae	White Clover	H	1600–3300	Europe	May–August	Banday et al. (2017)
155	*Vicia hirsuta* (L.) Gray	Fabaceae	Gray Vetch	C	800–1800	Eurasia	Mar–Apr	Rawat and Kharwal (2014)
156	*Vicia sativa* L.	Fabaceae	Common Vetch	H	Up to 1800	Eurasia	May–July	Rawat and Kharwal (2014)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
157	*Vicia sepium* L.	Fabaceae	Bush Vetch	H	Up to 1800	Eurasia	May–August	Banday et al. (2017)
158	*Corydalis cornuta* Royle	Fumariaceae	Horned Corydalis	H	2300–3600	Himalayan Region	July–Sept	Bisht (2017)
159	*Erodium cicutarium* (L.) Aiton	Gentianaceae	Common Stork's Bill	H	1700–2200	Africa and Europe	May–July	Banday et al. (2017)
160	*Gentiana cashemirica* Decne.	Gentianaceae	Kashmir Gentiana	H	2500–4000	Himalayan Region	May–July	Kumar et al. (2018)
161	*Geranium nepalense* Sweet	Gentianaceae	Nepal Geranium	H	1500–4000	Asia	May–Aug	Bisht (2017)
162	*Geranium wallichianum* D. Don ex Sweet	Gentianaceae	Cranesbill Geranium	H	2400–3600	Himalayan Region	May–Aug	Kumar et al. (2018)
163	*Swertia cordata* (Wall. ex D. Don) Clarke	Gentianaceae	Heart-Leaf Swertia	H	1700–4000	Himalayan Region	Sept–Oct	Bisht (2017)
164	*Hydrilla verticillata* (L.) Royle	Hydrocharitaceae	Water Thyme	H	Up to 1800	Asia and Africa	June–July	Kumar et al. (2018)
165	*Hypericum cernuum* Roxb.	Hypericaceae	Pendant St. John's Wort	H	1500–2600	Himalayan Region	May–Aug	Kumar et al. (2018)
166	*Hypericum perforatum* Buch-Ham. ex D. Don	Hypericaceae	Perforate St. John's Wort	H	1600–2900	Himalayan Region	May–July	Singh and Dangwal (2014)
167	*Hypericum uralum* L.	Hypericaceae	Nepal St. Johns Wort	H	1700–2600	Asia	April–Sept	Bisht (2017)
168	*Iris ensata* Thunb.	Iridaceae	Japanese Iris	H	1600-2200	Asia	April–May	Banday et al. (2017)
169	*Iris kumaonensis* Wall. ex D. Don	Iridaceae	Kumaon Iris	H	2400–4300	Himalayan Region	April–July	Kumar et al. (2018)

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Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
170	*Ajuga bracteosa* Wall. ex Benth.	Lamiaceae	Common Bugleweed	H	1200-3000	Africa and Asia	May–July	Bisht (2017)
171	*Ajuga parviflora* (Benth) Wall.	Lamiaceae	Small Flowered Bugleweed	H	1600–2800	Himalayan Region	May–June	John and Dube (1995)
172	*Colebrookia oppoitifolia* Sm.	Lamiaceae	Indian Squirrel Tail	S	300–1700	Asia	Apr–Nov	Rawat and Kharwal (2014)
173	*Leucas cephalotes* (Roth.) Spreng.	Lamiaceae	Head Leucas	H	150–2000	Asia	May–Sept	Dangwal et al. (2011)
174	*Leucas lanata* Benth.	Lamiaceae	Woolly Leucas	H	700–1800	Asia	Jan–Dec	Dangwal et al. (2011)
175	*Marrubium vulgare* L.	Lamiaceae	Common Horehound	H	1700–2500	Eurasia–Africa	June–Nov	Kumar et al. (2018)
176	*Mentha arvensis* L.	Lamiaceae	Corn Mint	H	1200–2600	Eurasia–Africa	May–August	Banday et al. (2017)
177	*Micromeria biflora* (Buch-Ham.) Benth.	Lamiaceae	Lemon Savory	H	1000–4000	Asia and Africa	May–August	Bisht (2017)
178	*Nepata cetaria* L.	Lamiaceae	Catmint	H	2200–3300	Eurasia	May- July	Banday et al. (2017)
179	*Nepata ciliaris* Benth.	Lamiaceae	White-Leaved Catmint	H	2300–3600	Himalayan Region	May–July	Bisht (2017)
180	*Nepata graciliflora* Benth.	Lamiaceae	–	H	2000–3300	Himalayan Region	May–June	Bisht (2017)
181	*Perilla frutrscens* (L.) Britt.	Lamiaceae	Beefsteak Plant	H	600–2400	Asia	May–August	Rawat and Kharwal (2014)
182	*Pogostemon benghalensis* (Burm.f.) Kuntze	Lamiaceae	Bengal Pogostemon	H	200–1500	Asia	Nov–Dec	Bisht (2017)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
183	*Prunella vulgaris* L.	Lamiaceae	Self-Heal	H	1600–3000	Africa and Europe	May–Aug	Ganie et al. (2016)
184	*Salvia lanata* Roxb.	Lamiaceae	Bush Sage	H	1000–1600	Eurasia	Mar–July	Bisht (2017)
185	*Salvia moorcroftiana* Wall. ex Benth	Lamiaceae	Moorcroft's Salvia	H	1800–2700	Himalayan Region	May–June	Banday et al. (2017)
186	*Scutellaria gaelericulata* L.	Lamiaceae	Scull-Cap	H	1600–2500	Eurasia	July–Aug	Banday et al. (2017)
187	*Teucrium quadrifarium* Buch.-Ham. ex D. Don	Lamiaceae	–	S	500–2400	Asia	July–Sept	Bisht (2017)
188	*Thymus serphyllum* L.	Lamiaceae	Wild Thyme	H	2400–4500	Europe	June–August	Banday et al. (2017)
189	*Lemna gibba* L.	Lemnaceae	Large Duckweed	H	Up to 1600	Europe	July–August	Varshney et al. (2007)
190	*Spirodela polyrrhiza* (L.) Sch.	Lemnaceae	Least Duckweed	H	1700–2200	Africa and Europe	July–August	Ganie et al. (2015)
191	*Utricularia aurea* Lour.	Lentibulariaceae	Golden Bladderwort	H	1500–2700	Asia–Australia	June–Aug	Ganie et al. (2015)
192	*Ophiopogon intermedius* D. Don	Liliaceae	Lily of the Valley	H	800–1600	Asia	June–Sept	Kumar et al. (2018)
193	*Tulipa stellata* Hook.	Liliaceae	Indian Tulip	H	1800–2500	Asia	April–May	Banday et al. (2017)
194	*Reinwardtia indica* Dumor.	Linaceae	Yellow Flax	S	300–2300	Asia	Feb–May	Bisht (2017)
195	*Viscum album* L.	Loranthaceae	Mistletoe	H	600–2600	Eurasia	April–May	Kumar et al. (2018)
196	*Ammania baccifera* L.	Lythraceae	Monarch Redstem	H	200–1000	Asia and Africa	May–June	Kabdal et al. (2014)

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Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
197	*Woodfordia fruticosa* (L.) Kurz.	Lythraceae	Fire Flame Bush	S	200–1800	Asia and Africa	March–June	Rawat and Kharwal (2014)
198	*Abutilon indicum* (L.) Sweet	Malvaceae	Indian Mallow	H	200–1200	Australia	August–Nov	Singh and Dangwal (2014)
199	*Hibiscus trionum* L.	Malvaceae	–	S	1600–2200	Africa	June–Sept	Kumar et al, 2018
200	*Malva neglecta* Wall.	Malvaceae	Dwarf Mallow	H	1600–2400	Asia and Africa	May–July	Banday et al, 2017
201	*Malva parviflora* L.	Malvaceae	Cheeseweed Mallow	H	1600–2400	Eurasia–Africa	May–Aug	Dangwal et al. (2011)
202	*Malva sylvestris* L.	Malvaceae	Common Mallow	H	1600–2200	Eurasia	May–Aug	Banday et al. (2017)
203	*Sida acuta* Burm. f.	Malvaceae	Common Wireweed	S	250–2700	South America	Sept–March	Singh and Dangwal (2014)
204	*Sida rhombifolia* L.	Malvaceae	Jelly Leaf	S	Up to 1500	South America	June–Nov	Singh and Dangwal (2014)
205	*Urena lobata* L.	Malvaceae	Congo Jute	S	200–1400	Asia Africa	June–Aug	Singh and Dangwal (2014)
206	*Marsilia quadrifolia* L.	Marsileaceae	Four-leaved Clover	F	Up to 1500	Tropical Region		Dangwal et al. (2012)
207	*Tinospora cordifolia* (Thunb.) Miers.	Menispermaceae	Heart-Leaved Moonseed	S	200–1200	Asia	July–Aug	Dangwal and Singh (2012)
208	*Nelumbo nucifera* Gaertn.	Nelumbonaceae	Sacred Water Lotus	H	Up to 1700	Asia and Africa	June–Aug	Bisht (2017)
209	*Boerhavia diffusa* L.	Nyctaginaceae	Spreading Hogweed	H	300–1200	Asia and Africa	June–Aug	Rawat and Kharwal (2014)

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Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
210	*Mirabilis jalapa* L.	Nyctaginaceae	Four O' Clock	H	Up to 2000	Africa	June–Sept	Rawat and Kharwal (2014)
211	*Epibolium hirsutam* L.	Onagraceae	Hairy Willow Herb	H	1000–3000	Eurasia	July–Aug	Banday et al. (2017)
212	*Epibolium laxum* Royle	Onagraceae	Lax Willow Herb	H	2200–3300	Himalayan Region	July–Aug	Bahaar and Bhat (2012)
213	*Oenothera rosea* Aiton	Onagraceae	Evening Primrose	H	1200–2000	South America	May–Sept	Melkhania and Singh (1983)
214	*Oxalis corniculata* L.	Oxalidaceae	Woodsorrel	H	300–2900	Europe	Feb–Nov	Banday et al. (2017)
215	*Oxalis latifolia* Kunth	Oxalidaceae	Simple Perennial Woodsorrel	H	300–2200	North America	Feb–Oct	Tiwari et al. (2016)
216	*Argemone mexicana* L.	Papaveraceae	Mexican Poppy	H	200–1500	North America	Feb–May	Tiwari et al. (2016)
217	*Papaver dubium* L.	Papaveraceae	Long Headed Poppy	H	1800–2700	Europe	April–May	Kumar et al (2018)
218	*Eurya acuminata* DC.	Pentaphylacaceae	Tapering Leaf Eurya	S	1500–2400	Asia Himalayan Region	May–June	Bisht (2017)
219	*Plantago lanceolata* L.	Plantaginaceae	Ribwort Plantain	H	1200–2200	Africa and Europe	May–Aug	Banday et al. (2017)
220	*Plantago major* L.	Plantaginaceae	Broadleaf Plantain	H	1200–2600	Eurasia	May–Aug	Banday et al. (2017)
221	*Aegilops tauschii* Coss.	Poaceae	Tausch's Goat Grass	H	1000–2000	Asia	April–June	Banday et al. (2017)
222	*Agrostis tenuis* Sibth.	Poaceae	Common Bentgrass	H	1900–2500	Europe	May–June	Banday et al. (2017)

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Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
223	*Alopseurus pratensis* L.	Poaceae	Meadow Foxtail	H	2000–2800	Eurasia	May–July	Ganie et al. (2015)
224	*Avena fatua* L.	Poaceae	Wild Oat	H	Up to 2000	Asia	April–June	Banday et al. (2017)
225	*Avena sativa L.*	Poaceae	Spring Wild Oat	H	1500–2000	South America	April–May	Tiwari et al. (2016)
226	*Brachiaria ramose* (L.) Stapf	Poaceae	Browntop Millet	H	Up to 1800	Asia and Africa	July–Sept	Kumari and Saini (2018)
227	*Bromus catharticus* Vahl.	Poaceae	Prairie Grass	H	Up to 1700	North America	Aug–Oct	Tiwari et al. (2016)
228	*Bromus mollis* L.	Poaceae	Soft Brome	H	1600–1800	North America	May–Sept	Banday et al. (2017)
229	*Carex cernua* Boott.	Poaceae	–	H	200–1300	Asia	April–June	Bahaar and Bhat (2012)
230	*Cynodon dactylon* (L.) Pers.	Poaceae	Dhoob Grass	H	Up to 3000	Africa	April–July	Banday et al. (2017)
231	*Drepanostachyum falcatum* Nees	Poaceae	Dwarf Bamboo	H	1500–3600	Himalayan Region	–	Tiwari et al. (2016)
232	*Echinochloa colona* (L.) Link	Poaceae	Jungle Rice	H	Up to 1900	South America	June–Aug	Chopra et al. (2013)
233	*Echinochloa crusgalli* (L.) Beauv.	Poaceae	Barnyard Millet	H	Up to 1900	South America–Europe	July–Sept	Banday et al. (2017)
234	*Heteropogon contortus* (L.) Beauv.	Poaceae	Black Spear Grass	H	400–2600	Africa and Asia	Jan–March	Dangwal et al. (2011)

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Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
235	*Imperata cylindrica* (L.) Beauv.	Poaceae	Cogon Grass	H	700–2400	Asia	July–Oct	Tiwari et al. (2016)
236	*Lolium temulentum* L.	Poaceae	Poison Darnel	H	800–1100	Europe	April–June	Singh and Dangwal (2014)
237	*Pennisetum flaccidum* Griseb.	Poaceae	Himalayan Fountain Grass	H	Up to 2400	Himalayan Region	May–Oct	Bahaar and Bhat. (2012)
238	*Saccharum spontaneum* L.	Poaceae	Wild Sugarcane	H	200–1800	Asia	July–Sept	Tiwari et al. (2016)
239	*Setaria glauca* (L.) *Beauv.*	Poaceae	Yellow Foxtail	H	1500–2200	Eurasia	Aug–Oct	Tiwari et al. (2016)
240	*Sorghum halepense* (L.) Pers.	Poaceae	Johnson Grass	H	Up to 1500	Eurasia–Africa	July–Aug	Banday et al. (2017)
241	*Sporobolus diander* (Retz.) Beauv.	Poaceae	Tussock Dropseed	H	1500–2000	Asia–Australia	May–July	Bisht (2017)
242	*Fagopyrum debotrys* (D. Don) Hara	Polygonaceae	–	H	1500–3400	Himalayan Region	Aug–Oct	Bisht (2017)
243	*Polygonum amphibium* (L.) Gray.	Polygonaceae	Amphibious Knotweed	H	2500–3800	Himalayan Region	May–July	Varshney et al. (2007)
244	*Polygonum hydropiper* (L.) Delab.	Polygonaceae	Water Pepper`	H	900–2400	Asia and Africa	July–Sept	Dangwal et al. (2012)
245	*Polygonum persicaria* L.	Polygonaceae	Lady's Thumb	H	2200–3500	Eurasia–Africa	Feb–Nov	Tiwari et al. (2016)
246	*Polygonum plebejum* R. Brown	Polygonaceae	Indian Knotweed	H	300–1800	Asia and Africa	Jan–Dec	Gupta et al. (2008)

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Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
247	*Rumex dentatus* L.	Polygonaceae	Sour Dock	H	Up to 1400	Eurasia–Africa	June–August	Tiwari et al. (2016)
248	*Rumex hastatus* D. Don	Polygonaceae	Sheep's Sorrel	H	1200–2600	Asia	May–Sept	Kumar et al. (2018)
249	*Rumex nepalensis* Spreng.	Polygonaceae	Nepal Dock	H	1200–4200	Eurasia	June–July	Banday et al. (2017)
250	*Portulaca oleracea* L.	Portulacaceae	Common Purslane	H	300–1600	South America	April–May	Banday et al. (2017)
251	*Potamogeton nodosus* Poir.	Potamogetonaceae	Long Leaf Pondweed	H	1600–2600	Eurasia	May–July	Ganie et al. (2015)
252	*Anagalis arvensis* L.	Primulaceae	Blue Pimpernel	H	300–2500	Eurasia	June–August	Banday et al. (2017)
253	*Primula denticulate* Smith	Primulaceae	Wild Primula	H	1600–2200	Himalayan Region	April–June	Kumar et al. (2018)
254	*Anemone obtusiloba* D. Don	Ranunculaceae	Himalayan Anemone	H	2000–3400	Himalayan Region	June–Sept	Tiwari et al. (2016)
255	*Anemone rivularis* Buch-Ham. ex DC.	Ranunculaceae	River Anemone	H	1600–400	Himalayan Region	June–Oct	Bisht (2017)
256	*Anemone vitifolia* Buch-Ham.	Ranunculaceae	Wild Flower	S	2000–3000	Asia	July–Sept	Kumar et al. (2018)
257	*Aquilegia pubiflora* Wall. ex Royle	Ranunculaceae	Fragrant Columbine	H	2500–3300	Himalayan Region	July–August	Kumar et al. (2018)
258	*Clematis barbellata* Edgew.	Ranunculaceae	Brown Clematis	S	3000–3400	Himalayan Region	June–Aug	Bisht (2017)

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Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
259	*Clematis montana* Buch-Ham. ex DC.	Ranunculaceae	Mountain Clematis	S	1600–4000	Asia	June–Aug	Dangwal and Singh (2012)
260	*Delphinium denudatum* Wall. ex Hk. & T.	Ranunculaceae	Wild Delphinium	H	1500–2700	Himalayan Region	June–August	Kumar et al. (2018)
261	*Ranunculus acris* L.	Ranunculaceae	Meadow Buttercup	H	1200–2500	Himalayan Region	May–July	Banday et al. (2017)
262	*Ranunculus arvensis* L.	Ranunculaceae	Corn Buttercup	H	1600–3200	Europe	April–June	Rawat and Kharwal (2014)
263	*Ranunculus diffusus* DC.	Ranunculaceae	Spreading Buttercup	H	1500–2200	Asia	April–Aug	John and Dube (1995)
264	*Ranunculus hirtellus* Royle	Ranunculaceae	Softly Hairy Buttercup	H	2500–3800	Asia	May–Sept	Bisht (2017)
265	*Ranunculus laetus* Wall. ex HK. T.	Ranunculaceae	Cheerful Buttercup	H	1600–2800	Europe	Mar–April	Rawat and Kharwal (2014)
266	*Ranunculus muricatus* L.	Ranunculaceae	Spiny Fruit Buttercup	H	1600–2700	Africa–Europe	Mar–June	Rawat and Kharwal, (2014)
267	*Ranunculus repens* L.	Ranunculaceae	Creeping Buttercup	H	1500–2500	Eurasia–Africa	April–June	Kumar et al. (2018)
268	*Ranunculus sceleratus* L.	Ranunculaceae	Celery-Leaved Buttercup	H	1200–2200	Europe	May–July	Dangwal et al. (2011)
269	*Ranunculus trichophyllus* Chaix ex Vill.	Ranunculaceae	Threat Leaf Crowfoot	H	2000–3000	Eurasia	May–July	Ganie et al. (2015)
270	*Thalictrum foliolosum* DC.	Ranunculaceae	Meadow Rue	H	1300–2800	Himalayan Region	June–Oct	Tiwari et al. (2016)
271	*Thalictrum secundum* Edgew.	Ranunculaceae	–	H	1600–2800	Himalayan Region	June–Sept	Bisht (2017)

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Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
272	*Agrimonia pilosa* Ledeb.	Rosaceae	Hairy Agrimony	H	1000–3000	Eurasia	June–Aug	Bisht (2017)
273	*Cotoneaster microphyllus* Wall. ex Lind.	Rosaceae	Rockspray Cotoneaster	S	1500–3300	Himalayan Region	May–June	Bisht (2017)
274	*Duchesnea indica* (Andr.) Focke	Rosaceae	Indian Strawberry	H	Up to 2000	Asia	March–Sept	Tiwari et al. (2016)
275	*Fragaria nubicola* (Hook) L. ex Lacaila	Rosaceae	Wild Strawberry	H	1800–3800	Europe–Himalayan Region	June–Oct	Bisht (2017)
276	*Potentilla gerardiana* Lindl. ex Lehm.	Rosaceae	–	H	1500–2600		June–Sept	Bisht (2017)
277	*Potentilla nepalensis* Hook.	Rosaceae	Crimson Cinquefoil	H	2000–2800		June–Sept	Kumar et al. (2018)
278	*Potentilla reptans* L.	Rosaceae	Creeping Cinquefoil	H	1700–2600	Eurasia	June–Sept	Banday et al. (2017)
279	*Prinsepia utilis* Royle	Rosaceae	Himalayan Cherry Prinsepia	S	1600–2500	Himalayan Region	May–Aug	John and Dube (1995)
280	*Rosa brunonii* Lind.	Rosaceae	Himalayan Rusk	S	1200–2400	Africa–Europe	April–June	Bisht (2017)
281	*Rosa moschata* Herrm.	Rosaceae	Musk Rose	S	1500–2200	Asia	July–Aug	John and Dube (1995)
282	*Rubus ellipticus* Smith	Rosaceae	Yellow Himalayan Raspberry	S	1500–2400	Asia	May–July	Tiwari et al. (2016)
283	*Galium aparine* L.	Rubiaceae	Catch Weed	H	3000–4000	Africa–Europe	July–Aug	Bisht (2017)
284	*Galium asperifolum* Wall.	Rubiaceae	–	H	2700–3200	North America	June–Aug	Bisht (2017)
285	*Galium elegans* (Wall.) Roxb.	Rubiaceae	–	H	1600–2700	Africa–Europe	May–July	Bisht (2017)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
286	*Rubia cordifolia* L.	Rubiaceae	Indian Madder	C	1500–2500	Asia and Africa	July–Sept	Kumar et al. (2018)
287	*Azolla cristata* Kaulf.	Salviniaceae	Mosquito Fern	Pt	Up to 1700	North America		Ganie et al. (2015)
288	*Parnassia nubicola* Wall. ex Royle	Saxifragaceae	Himalayan Bog Star	H	2500–4000	Himalayan Region	July–Aug	Bisht (2017)
289	*Saxifraga diversifolia* Wall. ex Seringe	Saxifragaceae	Diverse-Leaved Saxifrage	H	2200–4800	Himalayan Region	July–Oct	Bisht (2017)
290	*Mazus japonicus* (Thunb.) Kuntze	Scrophulariaceae	Mazus	H	Up to 1500	Asia	July–Oct	Kumar et al. (2018)
291	*Scrophularia himalensis* Royle ex Benth.	Scrophulariaceae	Himalayan Figwort	H	2000–3500	Himalayan Region	June–August	Bisht (2017)
292	*Verbascum thapsus* L.	Scrophulariaceae	Great Mullein	H	1800–3600	Eurasia	July–Aug	Tiwari et al. (2016)
293	*Veronica anagallis* L.	Scrophulariaceae	Brook Pimpernel	H	1800–3000	Eurasia	May–June	Banday et al. (2017)
294	*Veronica arvensis* L.	Scrophulariaceae	Corn Speedwell	H	2200–3200	Europe	April–Oct	Banday et al. (2017)
295	*Veronica beccabunga* L.	Scrophulariaceae	Brooklime	H	1200–2500	Europe–Africa	April–Aug	Bahaar and Bhat (2012)
296	*Veronica biloba* L.	Scrophulariaceae	Two-Lobed Speedwell	H	2300–4200	Asia	April–Aug	Gupta et al. (2008)
297	*Veronica persica* Poir.	Scrophulariaceae	Common Speedwell	H	1500–3000	Eurasia	April–Aug	Gupta et al. (2008)
298	*Smilax aspera* L.	Smilacaceae	Rough Bindweed	C	Up to 1600	Africa Europe	July–Sept	Tiwari et al. (2016)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
299	*Smilax glaucophylla* Klotz.	Smilacaceae	Elegant Smilax	C	1000–2200	Asia	April–May	Tiwari et al. (2016)
300	*Datura metal* L.	Solanaceae	Datura	S	1500–2200	South America	July–Sept	Kumar et al. (2018)
301	*Datura stramonium* L.	Solanaceae	Jimson Weed	H	1600–2700	North America	June–Sept	Banday et al. (2017)
302	*Nicandra physalodes* (L.) Gaertn.	Solanaceae	Apple of Peru	S	800–2300	South America	May–Nov	Kumar et al. (2018)
303	*Solanum nigrum* L.	Solanaceae	Black Nightshade	H	1200–2500	South America	June–Oct	Banday et al. (2017)
304	*Solanum xanthocarpum* Schrad & Wendl.	Solanaceae	Yellow Fruit Nightshade	H	1500–2000	Asia	August–Oct	Dangwal et al. (2011)
305	*Corchorus aestuans* L.	Tiliaceae	East Indian Mallow	H	Up to 2000	Asia	Aug–Oct	Singh and Dangwal (2014)
306	*Triumfetta annua* L.	Tiliaceae	–	H	Up to 2200	Africa and Asia	June–Aug	Bisht (2017)
307	*Triumfetta pilosa* Roth.	Tiliaceae	Diamond Burbark	H	1000–2600	Asia and Africa	June–Aug	Bisht (2017)
308	*Trapa natans* L.	Trapaceae	Water Chestnut	H	1600–2200	Eurasia	July–Nov	Varshney et al. (2007)
309	*Girardinia diversifolia* (L.) Friis	Urticaceae	Himalayan Nettle	H	500–1600	Himalayan Region	Sept–Oct	Singh and Dangwal (2014)
310	*Pouzolzia indica* (L.) Gaudich	Urticaceae	Graceful Pouzolz's Bush	H	300–1500	Asia	July–Oct	John and Dube (1995)
311	*Pouzolzia pentandra* (Roxb.) Benn.	Urticaceae	Melastome Pouzolz's Bush	H	Up to 1200	Asia	July–Oct	Dangwal et al. (2012)
312	*Urtica dioica* L.	Urticaceae	Common Nettle	H	1600–3000	Africa–Europe	June–Sept	Bisht (2017)

(continued)

Table 1.1 (continued)

S. no.	Taxa	Family	Common name	Life form	Range of altitude (m)	Nativity	Flowering period	References
313	*Urtica parviflora* Roxb.	Urticaceae	–	H	1500–2800	Himalayan Region	May–June	John and Dube (1995)
314	*Valeriana hardwickii* (Wall.) Roxb.	Valerianaceae	–	H	1900–3100	Asia	May–July	Bisht (2017)
315	*Valeriana jatamansi Jones*	Valerianaceae	Indian Valerian	H	1500–3600	Himalayan Region	May–July	Tiwari et al. (2016)
316	*Lantana camara* L.	Verbenaceae	Wild Sage	S	Up to 2000	South America	April Sept	Angiras, 2014
317	*Viola biflora* Smith	Violaceae	Arctic Yellow Violet	H	2300–4300	Eurasia–North America	May–June	Bisht (2017)
318	*Viola canescens* Wall. ex Roxb.	Violaceae	Dog Violet	H	2500–4300	Asia	April–May	Kumar et al. (2018)
319	*Viola pilosa* Blume	Violaceae	Smooth Leaf White Violet	H	2200–4300	Asia	March–April	Bisht (2017)
320	*Viola tricolor* L.	Violaceae	Wild Pansy	H	1500–1800	Europe	March–May	Bisht (2017)
321	*Vitis lanata* Roxb.	Vitaceae	Wild Grape	C	900–2100	Eurasia	June–July	Tiwari et al. (2016)
322	*Hedychium spicatum* Smith	Zingiberaceae	Spiked Ginger Lily	H	1800–2800	Himalayan Region	July–August	Kumar et al. (2018)
323	*Tribulus terrestris* L.	Zygophyllaceae	Bullhead	H	1900–3000	Africa–South America	July–August	Dangwal et al. (2011)

H Herb, *S* shrub, *C* climber, and *Pt* pteridophytes/Fern

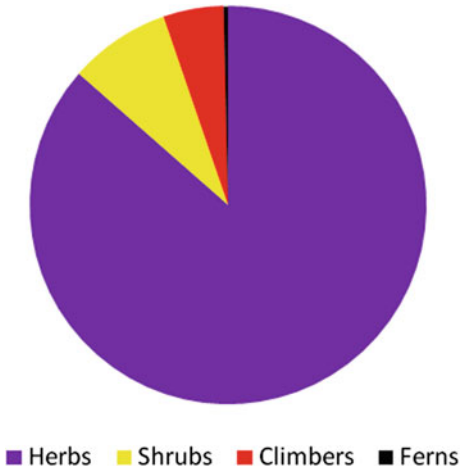

Fig. 1.1 Diversity of life forms of weed species

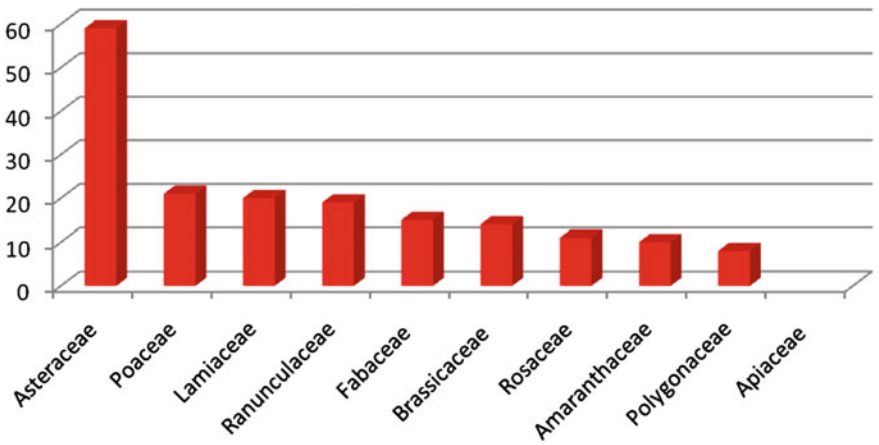

Fig. 1.2 Dominant families of weed species

yield. Further, the flowering period of weeds coincides with the flowering period of crops. Weeds mature ahead of crops so that their seeds get mixed with crop seeds, thus resulting in adulteration, and these weeds compete again with the crops in the next season, so the battle against weeds is a never ending one (Tiwari et al. 2016). Weeds cause many billion of crop losses annually, and identification and proper management of weeds will reduce the loss by increasing crop yield and quality.

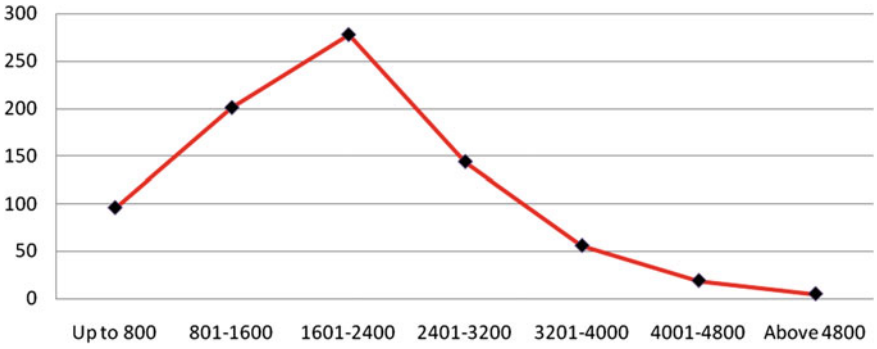

Fig. 1.3 Altitude-wise distribution pattern of weed species

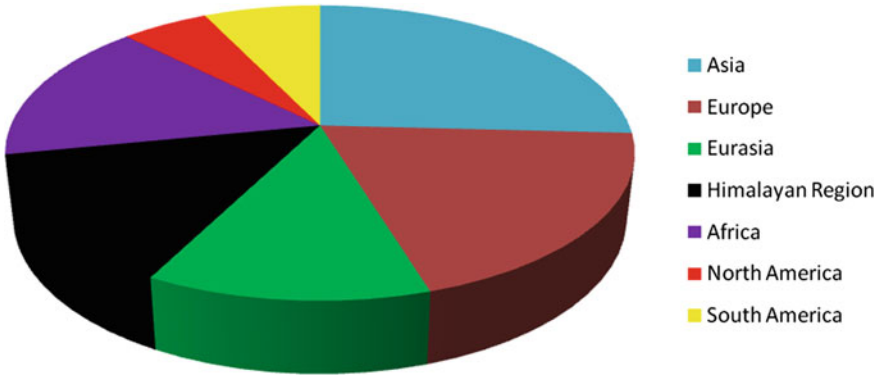

Fig. 1.4 Distribution of native and non-native weed species

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Part II
Bioremediation

Chapter 2
Utilization of Aqueous Weeds for Biofuel Production: Current Status and Future Prospects

Rafiq A. Rather and Madhulika Bhagat

2.1 Introduction

The energy crisis is one of the biggest issues encountered by today's world. Rapid economic growth and population explosion have resulted in a substantial increase in energy (fuel) consumption, especially in the transportation sector. The currently available sources of energy (such as fossil fuels) are limited and are consumed at an alarming rate throughout the world. Such limited sources of energy are likely to get exhausted over time. Therefore, there is a need to develop new technologies of harnessing alternative, sustainable and eco-friendly sources of fuel energy (Coyle 2007; Luque et al. 2008). While at present most energy crops are depriving human feedstock, fermentation of fast growing aquatic weeds possesses an excellent prospect to become a significant source for biofuel, as both substrates are widely acquirable and do not need agriculturally arable land (Busic et al. 2018). Aquatic weeds include all those unabated plants which grow and complete their life cycle in water bodies and directly cause damage to aquatic ecosystems (Priya et al. 2014). They can easily grow and propagate in eutrophicated and polluted aquatic ecosystems. Aquatic weeds can be divided into three categories: floating, submerged, and emergent categories, all of which can potentially interfere with the standing and flowing water systems. Water hyacinth, water lettuce duckweed, azolla, etc., are common examples of floating aquatic weeds; whereas, hydrilla, eel grass, curly-leaf pondweed, coontail, parrot feather, elodea, etc., belong to submerged weeds. The emergent weeds include water lily, water chestnut, water willow, water primrose, water shield, alligator weed, cattail, purple loosestrife, water pod, spike rush, pickerelweed, smart weed, pickerel weed, soft rush, etc.

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Aquatic weeds found in the aquatic ecosystems represent a potential feedstock for production of biofuel (Borah et al. 2019). Biofuels are often defined as the solid, liquid, or gaseous fuels produced from the biomass of plants or biodegradable portion of the plant-derived materials (Giampietro et al. 1997; Webb and Coates 2012). Because biofuels are produced from the plant-derived polysaccharides (starch, cellulose, hemicellulose etc.), atmospheric CO_2 does not increase when biofuels are combusted, which refers to the concept of carbon neutrality (DeCicco 2018). Thus, utilization of biofuels in place of fossil fuels is an effective way to combat the global climate change by reducing greenhouse gas (GHG) emission and decreasing the dependence on limited sources of fossil fuels (Luque et al. 2008; Tilche and Galatola 2008). Because of their aggressive growth, aquatic weeds are often considered as invasive plants in most countries. Therefore, it is believed that their utilization and use as renewable energy source for biofuel production may significantly mitigate their deleterious effects on environment and contribute to their dilution and control. Different conversion technologies, genetic, and computational approaches are currently in use to convert weed biomass into biofuel (Koussa et al. 2014; Misra et al. 2013). Different aquatic weeds have different biofuel producing potentials (Abbasi et al. 1990). The main biofuel produced and consumed in the USA is bioethanol. The major sources of bioethanol today are sugar cane (especially in Brazil) and corn (particularly in the USA) (Low et al. 2007). The production of biofuels from aquatic weeds has recently garnered tremendous attention due to an abrupt increase in oil prices, depletion of existing fossil fuel reserves, and uninterrupted rise in greenhouse gas emission (Kaur et al. 2018b). Aquatic weeds exhibit exceptionally high vegetative reproduction rates and are rich in cellulose and hemicellulose with a very low lignin content that makes them an efficient biofuel feedstock (Armah-Agyeman et al. 2016; Haynes 1988; Kaur et al. 2018b). There are certain factors that limit the use of aquatic weeds for biofuel production. For example, microorganisms currently used in industrial bioreactors are incapable of efficiently fermenting pentose sugars present in the polysaccharide component of weed biomass, leading to low yield of biofuels (Elshahed 2010). Likewise, certain inhibitory compounds are also produced during the pretreatment of weed biomass, which may negatively influence the efficiency of enzymatic saccharification (breaking a complex carbohydrate into its monosaccharide components) and subsequent fermentation of digested biomass. These inhibitory compounds substantially reduce yield and productivity of bioprocessing (Conde-Mejia et al. 2012; Premjet 2018). Another important challenge faced during the commercial production of biofuel from aquatic weed biomass relates to the cost-effective breakdown of highly recalcitrant architecture of weed biomass into its fermentable sugars by various pretreatment methods (Elshahed 2010). Further, converting weed biomass into biofuel with the help of high end technology and costly instrumentation may limit their commercialization and industrial applicability, especially for developing countries. Moreover, surplus biomass bioprocessing in large-scale industrial bioreactors may not be economical due to the certain problems faced during collection, transportation, and infrastructural development. Therefore, an integrative and continuous effort is required to optimize the transformation of aquatic weed

biomass into biofuel. The production of biofuel from weed biomass is heavily influenced by government policies and subsidies, and any weakening of these policies is likely to influence biofuel production in several countries (Sorda et al. 2010; Su et al. 2015). This chapter describes the general perspectives for transformation of aquatic weed biomass into biofuel by integrating different pretreatment methods followed by the fermentation of the weed digest towards biofuel production.

2.2 Sources of Biofuels

Biofuels are often described as combustible fuels produced from biomass (Shalaby 2013). The biomass or organic matter that is converted to biofuels is derived from plants (including algae, weeds, trees, herbs, and crops) and synthesized via photosynthesis from CO_2 and water in the presence of chlorophyll (the green pigment in the chloroplast) and sunlight. Biofuels are produced from the biomass of recently living plant material as opposed to ancient fossilized plant material in fossil fuels. There are two main types of biofuels—bioethanol and biodiesel (Pinto et al. 2005). Biochemically, bioethanol is alcohol and biodiesel is oil. Ethanol is prepared by fermentation whereas biodiesel is produced by extracting naturally occurring oils from plants and seeds in a process called trans-esterification. Biofuel is usually used as a replacement for transportation fuels like diesel, petroleum, and jet fuels (Coyle 2007). Biofuel is an excellent alternative to environmentally harmful fossils fuel, as they can be produced in bulk from abundant supplies of renewable biomass with less greenhouse gas (GRG) emission. Environmentally, biofuels are described as carbon neutral, as the weed biomass sequesters roughly the same amount of carbon dioxide during growth and releases the same amount of CO_2 when combusted. Fossil fuels are used on a large scale in the world. However, because of their unsustainable use, they increase CO_2 levels causing accumulation of greenhouse gases which make the environment unhealthy. Recently, life cycle analysis (LCA) tool has been developed to evaluate biofuel and bioproduct sustainability (DeCicco 2018, Dunn 2019). The production of biofuel from the biomass of different aquatic weeds and agriculture wastes can be achieved by the application of a number of technologies, each having specific requirements, merits, and demerits (Busic et al. 2018; Swain 2014). Recently, the detection of environmental DNA (eDNA) using high-throughput sequencing has emerged as an important and effective tool for monitoring species diversity in aquatic ecosystems which may be useful in the identification of new aquatic weeds with potential biofuel feedstock characteristics (Kuzmina et al. 2018). The use of eDNA is believed to speed up the process of identification of aquatic weeds with desirable qualities for biofuel production. Here are some important sources of biofuels (Shalaby 2013).

2.2.1 Algae

Algae have recently gained the tremendous attention to be used as biofuel producers (Demirbas 2010; Pandey et al. 2019). Algae are usually small photosynthetic organisms that grow in aquatic environments and use sun light, atmospheric carbon dioxide (CO_2), and water to produce biomass. There are two major groups of algae that can be used for biofuel production: macroalgae and microalgae (Kaur and Yogalakshmi 2019). Macroalgae are usually large (measured in inches), multi-cellular algae that can be seen growing in aquatic environments. Microalgae, on the other hand, are tiny (measured in micrometers), unicellular algae that normally grow in suspension within aquatic ecosystems. Different types of algae have different growth characteristics, chemical compositions, and biofuel feedstock abilities. Normally algae have 20–80 % oil contents that could be converted into different types of biofuels such as biodiesel and biokerosene (Demirbas and Demirbas 2011; Khan et al. 2017). Carbohydrate rich algal biomass is also an excellent feedstock for biofuel production (Kaur and Yogalakshmi 2019). They are considered as the safer, non-competitive, and rapidly growing organisms compared to other aquatic plants that could be used for biodiesel production (Daroch et al. 2013; Georgianna and Mayfield 2012). The biodiesel production from algae is commercially economical and technologically not tedious (Demirbas and Demirbas 2011). Different species such as *tribonema, ulothrix* and euglena have immense potential for biofuel production (Khan et al. 2017). Algal biofuels do not contain environmentally deleterious chemicals, so environment can be kept safe and clean after their combustion. Algae can be metabolically engineered by manipulating these photosynthetic organisms to produce and secrete biofuels that may, in future, guarantee significant simplification of down-stream processing (Daroch et al. 2013). Recent advances in metabolic engineering and synthetic biology may play a vital role in biofuel production form algae by maximizing the metabolic output and stimulating the algal growth (Jagadevan et al. 2018).

2.2.2 Aquatic Weeds

Aquatic weeds belong to an important group of promising renewable energy source. Aquatic weeds possess high contents of starch, cellulose, and fatty acids and, therefore, the biomass of aquatic weeds can be efficiently and economically utilized as potential feedstock for production of ethanol, butanol, biodiesel, etc. (Kaur et al. 2018a; Murphy 1988). They can be easily hydrolyzed to fermentable sugars and constitute an efficient, cost-effective biofuel source compared to other lignocellulosic biomasses. Aquatic weeds have been grouped into classified into emergent, floating, and submerged weeds according to the various habitats (Jayan and Sathyanathan 2012), all of which can be carefully utilized for biofuel production.

2.3 Benefits of Aquatic Weeds as Effective Biofuel Feedstock

Aquatic weeds have long been regarded as an intriguing potential feedstock because of their following characteristics

2.3.1 Fast Growth Rate

Aquatic weeds naturally grow vegetatively at a very fast speed that can provide a continuous supply of cheap feedstock for biofuel production (Haynes 1988). Aquatic weeds usually multiply by vegetative propagation, including fragmentation, budding, and turion formation. For instance, an annual yield of water hyacinth (*Eichhornia crassipes*) is 100 dry tons/ha in natural lakes while the highest yields obtained for switchgrass (*Panicum virgatum*) in the USA is 25 dry tons/ha (Kaur et al. 2018a). Water hyacinth, due to its high vegetative growth, has excellent potential as a biofuel feedstock for bioethanol production (Sagar and Kumari 2013).

2.3.2 Unique Biochemical Composition

Aquatic weeds are rich in hemicelluloses, cellulose, fatty acids, and starch with relatively low lignin content. For example, water hyacinths are cellulose-rich with low lignin content that makes them an excellent feedstock for biofuel production (Sagar and Kumari 2013). Therefore, aquatic weeds can be easily hydrolyzed to fermentable sugars and constitute an efficient and cost-effective biofuel feedstock as compared to wood and other lignocellulosic biomass. The potential of aquatic weeds in biofuel production and their composition varies considerably between weed species (Gusain and Suthar 2017; Harper and Daniel 1934).

2.3.3 More Efficient Than Lignocellulosic Crops

Aquatic weeds provide more efficient feedstock for biofuel production in comparison to lignocellulosic (e.g. wood, grass) feedstock and do not need arable land, freshwater, or other agricultural requirements like energy crops (e.g. Switchgrass, Miscanthus) (Zabed et al. 2016). Converting aquatic weeds such as water chestnuts and hyacinths to biofuel could be an effective way to eliminate their detrimental effect on aquatic ecosystems and provide feedstock for much-needed energy (Lee and Fagan 2015).

2.3.4 Adaptability to Extreme Conditions

Aquatic weeds can grow quite well in severe environmental conditions that are otherwise detrimental to other plant growth. Due to their ability to overcome the seasonal restraints such as the varying temperature zones, biofuels can be derived from them continuously throughout the season.

2.3.5 Bioremediation Ability

Some aquatic weeds can be used dually for wastewater treatment and biofuel production. Application of aquatic weeds as potential candidates for bioremediation of polluted and contaminated environments is well-known facts during last few decades. For example, duckweeds, water lettuce, and water hyacinth have been extensively used in the bioremediation of polluted water in wastewater treatment systems (Vymazal and Kröpfelová 2008). Some of the most efficient aquatic plant species that are useful in removal of heavy metals from water bodies include water hyacinth, hornwort, curly-leaf pondweed, southern cattail, and common reed (El Falaky et al. 2004). Water hyacinth has been shown to efficiently absorb toxic heavy metals such as lead, mercury, copper, tin, and zinc from polluted water bodies (Priya et al. 2014).

2.3.6 Economical

Aquatic weeds usually reproduce through spores or vegetative propagation, thereby nullifying the seed cost. They can grow in wastewater at high and can be easily harvested (by mechanical harvesters or by skimming) at low cost, thereby decreasing the biofuel production cost (Oyedeji and Abowei 2012).

2.3.7 Efficient Utilization

Utilizing aquatic weed biomass as biofuel feedstock can provide an additional and cost-effective benefit of renewable energy generation while minimizing the economic and ecological damage caused by their rapid undesired growth. Usually, the utilization of aquatic weeds is preferred over their control as control of aqueous weeds is energy-dependent process where fossil fuels are used for herbicide production, equipment manufacturing, and machinery operation. Further utilization of aquatic weeds for biofuel production can have a dual benefit. Their growth can lead

to nutrient recovery (phytoremediation) from wastewater and conversion of nutrients into organic fertilizers (by-product) via anaerobic fermentation.

2.4 Advantages of Using Biofuels Over Fossil Fuels

Recently, extensive research has been carried out to develop alternative sources of biofuel production. Considering the problems associated with first-generation and second-generation biofuels, aquatic weed biomass can be considered as the everlasting next-generation biofuel resource. Despite having multiple benefits, the production and utilization of aquatic weeds as biofuel a feedstock are encountered by several issues that need to be resolved for efficient feedstock utilization. Nevertheless, there are several advantages of using biofuels as a replacement for fossil fuels:

- biofuels are renewable fuels and can potentially be produced continuously from weed biomass
- biofuels degrade at a faster speed than fossil fuels, thereby minimizing the ecological consequences of biofuel spills
- biofuel generation leads to relatively low emission of contaminants such as carbon monoxide, polycyclic aromatic hydrocarbons (PAHs), aldehydes, and particulate matter (PM)
- biofuel production from aquatic weeds is relatively safe to human health due to the minimal release of carcinogenic substances
- biofuel production is not associated with any emission of sulphur dioxide (SO_2)
- biofuels have relatively higher flashpoints (Ps) than the fossil fuels that make biofuels safe for storage, handling, and transportation
- biofuel exhibit more attractive properties, such as a higher specific energy, a higher cetane index, better lubricity, and enhanced cold flow behaviour

2.5 Aquatic Weeds Feedstock Evaluation

Because of the following characteristics of aquatic weed biomass, it can be used as a feedstock for generating biofuel

- ideal attributes
- wide availability
- ease of cultivation
- easy to process
- low tech processing
- inexpensive
- a huge amount of money spent each year on the disposal of aquatic weeds.

2.6 Biological Conversion of Aquatic Weed Biomass to Biofuel

The adverse effect of aquatic weeds on the aquatic environment is a serious concern worldwide, challenging the entire international community (Holm et al. 1977; Jayan and Sathyanathan 2012; Murphy 1988). However, careful reduction or elimination of aquatic weeds is possible through well-planned management strategies which include both preventive and control (biological, physical, chemical, eco-physiological) methods (Jayan and Sathyanathan 2012). All efforts to control the growth and spread of aquatic weeds have largely failed and hence the concept of "eradication of aquatic weeds through their utilization towards biofuel production" is being attempted. The great diversity and abundance of weed species provide a wide range of starting strains for enough biofuel production. Biofuel can be prepared from different components of aquatic weeds such as starch, sugars, cellulose, and lignocellulosic biomass (Murphy 1988). The most important step in the conversion of aquatic weed biomass to biofuel is the pretreatment of biomass to make it more digestible and accessible to enzymes and microorganisms in the subsequent steps. The cellulose, hemicelluloses, and other polysaccharides are then hydrolyzed to monomer sugars (pentoses and hexoses) followed by fermentation of these monomer sugar molecules to biofuel (Premjet 2018). Subsequently, biofuel is purified through distillation or other processes such as dehydration to world biofuel specifications (Fig. 2.1).

2.6.1 Pretreatment of Weed Biomass

The resistance of plant cell walls to deconstruction of aquatic weed biomass is a major problem in the conversion of aquatic weed biomass to biofuel (Premjet 2018; Zhao et al. 2012). This property of weed biomass is known as recalcitrance and is due to the highly crystalline and compact structure of plant cell wall. Biochemically, plant cell wall is composed of cellulose coupled with lignin and hemicellulose which are strongly bonded to each other (Hall et al. 2010; Zgórska 2016).Thus, recalcitrance of weed biomass is mainly attributed by its biochemical architecture that forms a protective barrier against biodegradation (Himmel et al. 2007; Zgórska 2016). Therefore, pretreatment of weed biomass is critical in releasing fermentable sugars from weed biomass for biofuel production. It is believed that pretreatment helps to cleave the bonds between lignin and hemicelluloses, and hence breaks the protective cover of cellulose (Lee and Fagan 2015). It also helps to decrease cellulose crystallinity making it more susceptible to enzymatic hydrolysis and fermentation (Hendriks and Zeeman 2009). Different pretreatment methods have been developed that can be used on various types of weed biomasses for biofuel production. Different types of aquatic weeds may need different set of pretreatment methods for efficient biofuel production. The ultimate aim

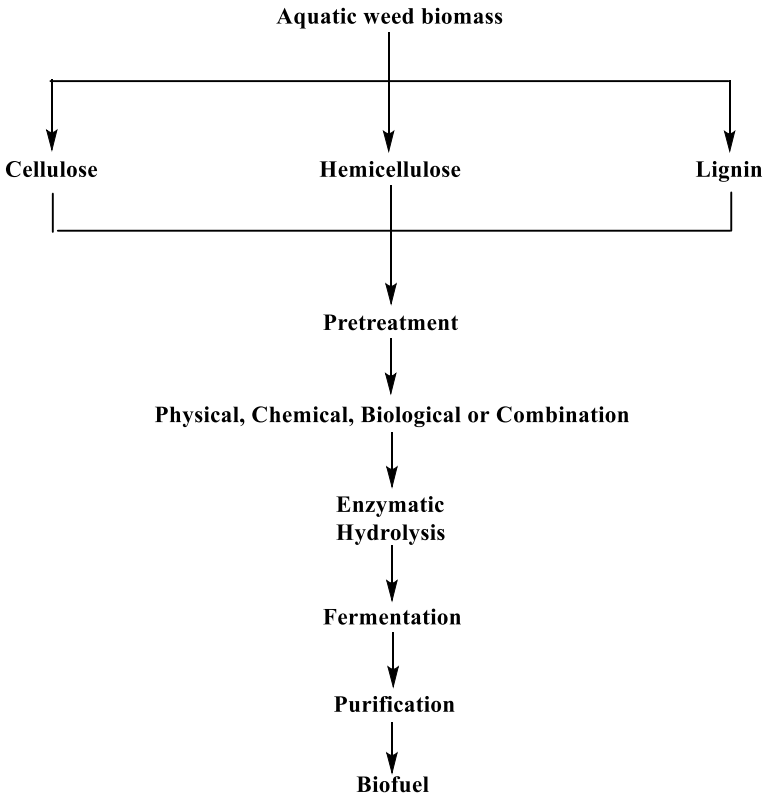

Fig. 2.1 Schematic representation of the conversion of weed biomass to biofuel

of all forms of pretreatment methods is to break the crystalline structure of cellulose, remove lignin and increase the porosity of the weed biomass (Kumar et al. 2009).

2.6.1.1 Physical Pretreatment of Aquatic Weeds

Physical pretreatment is an important step, or a group of steps, in the production of biofuels, comprising harvesting, transport, storage, washing, size reduction, drying, and compacting (Gaurav et al. 2017). In physical pretreatment, the biomass is mechanically comminuted into particles of smaller size, or the surface area of the biomass is increased without size reduction. The mechanical combinations of weed biomass can be achieved by grinding, milling, or chipping (Kapdan and Kargi 2006). Other methods of physical pretreatment include different kinds of irradiation pretreatment, pyrolysis, and ultrasonic pretreatment. All these methods have been developed to physically enhance accessibility of cellulose to enzymes and

microorganisms during the conversion process (Arsène et al. 2017; Passos et al. 2015). The effect of different physical pretreatment on the release of sugars from water hyacinth has been extensively studied and it has observed that different pretreatment methods cause different amounts of structure disruption and sugar release from biomass (Ganguly et al. 2012; Harun et al. 2011). Physical pretreatment usually disrupts the lignocellulosic structure with little or no chemical modifications to the individual cell wall components. An important drawback of physical pretreatment is its inability to remove the lignin, which limits access of cellulose to enzymes and microorganisms during fermentation. Lignin is a kind of "glue" that binds the different components of cellulose and hemicellulose. Other drawbacks of physical pretreatment pertain to its high energy consumption, the prohibitive costs of its large-scale implementation, and the huge environmental and safety concerns associated with it (Alvira et al. 2010; Sun and Cheng 2002). Recent studies have shown that delignification of the weed biomass could be the reason for the high energy consumption of physical pretreatment methods, and as such, physical pretreatment can ultimately affect the overall energy efficiency of a biorefinery (Kumar et al. 2009).

2.6.1.2 Chemical Pretreatment of Aquatic Weeds

Chemical pretreatment is the most common method for the conversion of aquatic weed biomass into biofuel (Bensah and Mensah 2013; Thi et al. 2017). Some of the most promising chemical pretreatment methods used for pretreatment of aquatic weeds involve the application of different types of chemicals such as alkalies, ionic liquids, organic solvents, oxidizing agents, and acids (Conde-Mejia et al. 2012). For example, acid pretreatment involves the use of various types of acids such as sulphuric acid, nitric acid, hydrochloric acid, oxalic acid, formic acid, acetic acid, ormaleic acids to remove hemicellulose components and expose cellulose for enzymatic digestion. Acidic pretreatment causes solubilization of hemicelluloses but has a relatively low effect on delignification (Conde-Mejia et al. 2012; Thi et al. 2017). The efficiency of acidic pretreatment is often determined by the type of acid, acid strength, concentration, volume, and pretreatment temperature. Usually, dilute acid is preferred over concentrated acid because concentrated acid is toxic, corrosive, and often leads to the production of the high level of inhibitors such as furfural derivatives, acetic acid, phenolics, and other aromatic compounds. Acidic pretreatment of water hyacinth with dilute (2 %v/v) sulphuric acid over a 20 min reaction time yielded higher concentration of reducing sugars than concentrated sulphuric acid (Reales-Alfaro et al. 2013). Acidic pretreatment may be conducted at low temperature for a longer period of time or at high temperature for a short time. Despite its effectiveness, acid pretreatment is toxic and generates inhibitory compounds that negatively affect enzymatic hydrolysis and fermentation processes. It is, therefore, crucial to remove these compounds, a process that adds to the cost of bioethanol production.

Alkali pretreatment refers to the application of alkaline solutions to remove lignin and various uronic acid substitutions on hemicellulose that lower the accessibility of enzymes to the hemicellulose and cellulose (Conde-Mejia et al. 2012). Alkaline pretreatment breaks the intermolecular bonds between lignin and hemicelluloses and substantially reduces cellulose crystallinity. Alkali pretreatment may be carried out using various types of alkalies such a sodium, calcium, ammonium, and potassium hydroxides at varying temperatures with or without pressure. Alkaline hydrolysis of water hyacinth with sodium hydroxide (NaOH) produces a mixture of sugars with xylose as the most abundant component (Kumari et al. 2014). Alkali pretreatment facilitates the accessibility of enzymes to cellulose by solubilizing lignin and causes relatively low sugar degradation and production of inhibitors compared to acid pretreatment.

Ozone, a very potent oxidizing agent, plays an important role in breaking down lignin, leading to biomass destruction, and delignification (Conde-Mejia et al. 2012). Ozone mediated chemical pretreatment, or ozonolysis is usually performed at room temperature and results in production of little or no inhibitors (Capolupo and Faraco 2016; Travaini et al. 2016). Many other organic solvents including methanol, ethanol, ethylene glycol, glycerol, acetic acid, formic acid, phenol, and dioxane are also very effective in extracting lignin and hemicelluloses. Organic solvent pretreatment has an inherent ability to fractionate biomass into lignin, cellulose, and hemicellulose components with high purity (Zhang et al. 2016a). Recently, ionic liquids including cholinium cations and linear carboxylate anions have garnered much attention as promising solvents for pretreatment of biomass because of their ability to solubilize lignin and promote subsequent enzymatic saccharification (Ninomiya et al. 2013). An important advantage of the use of ionic liquids is the recovery of separate lignin and carbohydrate fractions after pretreatment (da Costa Lopes et al. 2013). However, ionic liquids are costly and can inhibit enzymatic hydrolysis and fermentation steps.

2.6.1.3 Biological Pretreatment of Aquatic Weed Biomass

Biological pretreatment involves the use of microorganisms such as bacteria, fungi, and actinomycetes that specifically degrade the complex three-dimensional structure of lignin and hemicelluloses (Maurya et al. 2015). These microorganisms are capable of secreting ligninolytic enzymes such as peroxidases (lignin peroxidase and manganese peroxidase) and laccases (Premjet 2018; Sindhu et al. 2016). Both classes of these enzymes are necessary for delignification of biomass (Baruah et al. 2018). The most important microorganism used during biological pretreatment is filamentous fungus (Zheng et al. 2012). White-rot fungi, and many other basidiomycetes, have also been widely used for the biological pretreatment of biomass (Müller and Trösch 1986; Rouches et al. 2016; Wang et al. 2014). A plethora of white-rot fungi such as *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Cyathus stercoreus*, *Pycnoporus cinnabarinus*, *Ceriporia lacerata*, and *Ceriporiopsis subvermispora* are able of producing lignin-modifying enzymes for

the effective delignification of weed biomass (Müller and Trösch 1986). Biological pretreatment is usually preferred over other methods of pretreatment because it does not produce toxic inhibitors, is more eco friendly, and requires low energy compared to other pretreatment strategies (Capolupo and Faraco 2016, Mielenz 2015; Ummalyma et al. 2019). However, the biological pretreatment is a very slow process and needs highly controlled conditions as well as large space, making it an unattractive option for the commercial production of biofuels. Furthermore, certain microorganisms non-specifically degrade cellulose and hemicelluloses in addition to lignin. To enhance the efficiency of biological pretreatment, more basidiomycetes fungi should be investigated for enhanced and selective delignification activity (Kumar and Sharma 2017). Recently, ligninolytic enzyme extracts or enzyme cocktails have been used for biological pretreatment that prevents non-specific break down of celluloses and hemicelluloses associated with microbial pretreatment (Ummalyma et al. 2019). These enzymes are isolated from lignin-modifying microorganisms (Janusz et al. 2017). Interestingly, crude enzyme extracts are known to contain certain proteins and mediators that stimulate the enzymatic activity of these enzymes, making crude enzyme extracts more effective than purified enzyme cocktails (Ummalyma et al. 2019). The major issue with enzymatic degradation of lignin is unavailability of sufficient amounts of active enzymes. The influence of biological pretreatment on weed biomass may vary depending upon the composition of weed biomass and accessibility of weed biomass to microbial or enzymatic action. Therefore development of optimal pretreatment conditions is essential for converting aquatic weeds such as water hyacinth into good quality biofuel (Ummalyma et al. 2019; Zhang et al. 2016b). Researchers usually use different set of pretreatment condition on different types of aquatic weeds to ensure the optimum release of fermentable sugars from aquatic weeds.

2.6.1.4 Physico-Chemical Pretreatment of Aquatic Weed Biomass

The physico-chemical pretreatment comprises of both physical and chemical processes to facilitate the digestibility of the weed biomass for subsequent enzymatic hydrolysis and fermentation (Baruah et al. 2018). The well-known physico-chemical pretreatment includes liquid hot water (LHW), steam explosion (SE), ammonium fibre explosion (AFEX), soaking in aqueous ammonia (SAA), and irradiation-chemical method.

***Steam-Explosion* (*SE*)**

Steam explosion pretreatment is a hydrothermal process and uses both chemical and physical techniques to break the dense three-dimensional structure of the weed biomass (Kumar 2013). In this pretreatment method, weed biomass is exposed to saturated steam at high pressure (0.5–4.8 MPa) for a short duration of time (60 min) followed by an abrupt decrease in pressure which results in disintegration of biomass into component fibre and fibre bundles. The disruption of the fibrils enhances the accessibility of the cellulose to cellulases and enzymes during hydrolysis.

Liquid Hot-Water (LHW) Pretreatment

This hydrothermal process involves the exposure of biomass to liquid water at elevated temperatures (150–240 °C) and short times (ranging from a few minutes up to an hour) in order to break the three-dimensional structure of biomass (Kumar 2013). The basic purpose of this process is to ensure complete solubilization of hemicellulose and separate it from the rest of the solid material while reducing the formation of inhibitors that inhibit the growth of fermentative microbes (Kim et al. 2009). This type of pretreatment causes pressurized water to rupture the cell structure, resulting in separation of biomass into two distinct products streams-*liquid hydroxalate*, containing hemicelluloses sugars, minerals, and degradation products such as furfural and acetic acid, and a *solid fraction* comprising most of the cellulose and lignin and some residual hemicelluloses.

Ammonium Fibre Freeze Explosion (AFEX)

AEX is a physicochemical process very similar to steam explosion pretreatment in which weed biomass is exposed to liquid anhydrous ammonia (NH_3) under high pressures (6.5–45 bar) and moderate temperatures for about 5–30 min, and then rapidly depressurized. The moderate temperatures (60–100 °C) used in this method are significantly lower than that of the steam explosion process (Dale and Moreira 1982; Maurya et al. 2015). The degree of disintegration of weed biomass during this process depends highly on the temperature because temperature strongly influences the pace of the ammonia vapourization within the bioreactor during depressurization. AFEX leads to the removal of lignin and some hemicelluloses, in addition to the decrystallization of cellulose, partly due to the strong affinity of ammonia for such biomass components (Maurya et al. 2015). AFEX does not solubilize hemicelluloses but does require recovery of ammonia for cost and environmental reasons.

Soaking in Aqueous Ammonia (SAA)

SAA involves treatment of biomass with aqueous ammonia (5–50 %w/w) at low temperatures (25–90 °C) under ambient pressure in a batch reactor. Pretreatment is undertaken for residence times ranging from about 1 h to 3 months. The main effect of ammonia treatment of biomass is delignification without significantly affecting the carbohydrate contents. It is a very effective pretreatment method especially for substrates that have low lignin contents such as agricultural residues and herbaceous feedstock. The ammonia-based pretreatment is well suited for simultaneous saccharification and co-fermentation (SSCF) because the treated biomass retains cellulose as well as hemicellulose. There are two different types of pretreatment methods based on aqueous ammonia: (1) high severity, low contact time process (ammonia recycle percolation; ARP), (2) low severity, high treatment time process (soaking in aqueous ammonia; SAA). Both these methods have merits and demerits.

Irradiation-Chemical Pretreatment

Irradiation-chemical pretreatment involves soaking of weed biomass in a solvent (water, acid, or alkali) and subsequent irradiation with microwaves, gamma

radiation, proton and electron beam, or radiations of radio frequency. However, in some cases, biomass is irradiated before the chemical or other pretreatment methods, which may have several advantage that include solubilization of lignin and hemicellulose, minimization of cellulose degradation, use of lower doses of chemical, and less severe conditions.

2.6.2 Enzymatic Hydrolysis

After pretreatment, aquatic weeds such as water hyacinths are subjected to acidic or enzymatic hydrolysis to cleave cellulose and hemicelluloses into fermentable sugars such as glucose and xylose (Mukhopadhyay et al. 2008; Premjet 2018). Enzymatic hydrolysis is eco-friendly process and is often preferred over acid mediated hydrolysis (Gupta et al. 2017). The total quantity of fermentable sugars released from biomass digest is determined by the composition of weed biomass and efficiency of pretreatment method (Liu et al. 2019) and accordingly different aquatic weeds may produce varying amounts of fermentable sugars (Ganguly et al. 2013). Enzymatic hydrolysis of weed biomass can be done in multiple ways. For example, pretreated weed biomass may be first hydrolyzed by enzymes and then fermented to produce biofuel. This process is known as *separate hydrolysis, and fermentation* (SHF) and as such requires two distinct set of conditions for enzymatic hydrolysis and fermentation (Yang et al. 2014). A major limitation of this process is the accumulation of high sugar levels which can inhibit enzyme activities. The enzymatic release of monomer sugars and their fermentation can also be carried out together through a process known as *Simultaneous Saccharification and Fermentation* (SSF) (Olofsson et al. 2008). The tendency of the accumulation of monomer sugars is less in this method because the released sugar molecules are simultaneously fermented to produce biofuel. The major drawbacks of this method are the need to find favourable conditions (e.g. temperature and pH) for both the enzymatic hydrolysis and the fermentation and the difficulty to recycle the fermenting microorganisms and the enzymes. Another important method of biofuel production is *Consolidated Bioprocessing* (CBP) in which a microorganism or group of microorganisms are used to convert biomass into biofuel in one step (Mbaneme-Smith and Chinn 2015; Yang et al. 2014). The microorganisms are capable of secreting saccharolytic enzymes that degrade biomass and ferment released sugars to bioethanol. Consolidated bioprocessing (CBP) of weed biomass to biofuel integrates all steps of enzyme production, saccharification, and fermentation biologically in one reactor. This method is very promising, and has been a subject of intense research effort during the recent years (Salehi Jouzani and Taherzadeh 2015).

Cellulase enzymes have been extensively used in the biofuel industries. These enzymes catalyze the hydrolysis of cellulose (Wilson 2009). The cellulose enzymatic system consists of three main components, namely endoglucanase

(1,4-β-D-glucan glucanohydrolase, EC 3.2.1.3), exoglucanase (1,4-β-D-glucan cellobiohydrolyase, EC 3.2.1.91), and β-glycosidase (cellobiase; EC 3.2.1.21) that effectively convert cellulosic substrates into fermentable sugars(Ladisch et al. 1983). Endoglucanase cleave cellulose into units of glucose, cellobiose, and cellotriose while exoglucanase cut it into cellobiose units. β-glycosidase, on the other hand, cleave cellobiose units into glucose that can be fermented to biofuel. Cellulase enzyme activity is highly dependent on the concentration, dosage, pH, temperature, and source of enzyme system. Composition of the weed biomass as well as the type of pretreatment technique used also influences the activity of cellulose enzyme system. Therefore, high hydrolysis efficiency can be achieved by using optimized temperature, time, pH, enzyme concentration, and biomass concentration.

The hemicellulose component of weed biomass may also be hydrolyzed with hemicellulases (such as endo-xylanase, exo-xylanase, and β-xylosidase) and auxiliary enzymes (such as α-arabinofuranosidase, α-glucuronidase, acetyl xylan esterase, and ferulic acid esterase) into monomer sugars for efficient fermentation to bioethanol (Gao et al. 2011; Premjet 2018). The hydrolysis of hemicelluloses is usually more complex than cellulose due to its heteropolymeric composition (mixture of pentoses and hexoses). Enzymatic cocktails containing both cellulases and hemicellulases have been extensively used to hydrolyze various pretreated weed biomass for biofuel production

2.6.3 Fermentation

After enzymatic hydrolysis is achieved, the supernatant containing various weed sugars (pentoses and hexoses) are subjected to fermentation (Patinvoh and Taherzadeh 2019). Different types of fungi and bacteria have been used to ferment sugars released from weed biomass to biofuel. *Zymomonas mobilis*, *Kluyveromyces* sp., and *Saccharomyces cerevisia* are well known for their ability to ferment weed sugars into biofuel. Similarly, *Pachysolen tannophilus*, *Pichia stipitis*, and *Candida shehatae* are utilized to ferment xylose to bioethanol. *S. cerevisiae* is the most common microorganism for commercial bioethanol production. For example, *S. cerevisiae* has been found very useful in the production of bioethanol from aquatic weeds such as water hyacinth and water lettuce (Anker et al. 2016; Soda et al. 2013). However, the growth and activity of *S. cerevisiae*during fermentation process are markedly inhibited by high temperature, osmotic stress, bioethanol concentration, and contamination from bacteria, thus affecting the yield of bioethanol production. Furthermore, the inability of *S. cerevisiae* to ferment pentoses substantially reduces the biofuel yield during the fermentation process.Therefore, there is a need to isolate and identify new strains of *S. cerevisiae* that are able to tolerate these conditions to improve biofuel production during fermentation. Certainstrains from *Pichia* sp., *Candida* sp., *Schizosaccharomyces* sp. and *Pachysolen* sp. have also been identified for fermentation of pentoses to bioethanol.

Fermentation of weed biomass to bioethanol is normally carried out in a bioreactor through three different modes of fermentation, i.e. batch, fed-batch, and continues. During batch fermentation of bioethanol production, the fermentation ingredients including substrate, culture medium, and nutrients are fed to bioreactor at the beginning of process and no feeding is done till the process is over and bioethanol is harvested (Dombek and Ingram 1987). However, during continuous fermentation process, the substrate, medium, and nutrients are fed to the bioreactor continuously and bioethanol harvested continuously (Lindskog 2018). The fed-batch fermentation is combination of the batch and continuous processes (Cheng et al. 2009). In this process, the fermentation ingredients are continuously added to the bioreactor but biofuel is harvested only at the end of the process. After harvesting, biofuel is purified through distillation or other processes to obtain commercial quality biofuel.

2.7 Biofuel Production in the United States

The USA is the largest producer of biofuels in the world and produces biofuel mainly in the form bioethanol and biodiesel. Other world's largest biodiesel producers are Brazil and the European Union. During the year 2006, the USA generated 4.855 billion US gallons of bioethanol and overtook Brazil as the world's largest bioethanol producer. In 2015, the USA together with Brazil produced approximately 70 % of global biofuel supply, with total world production of 13.5 billion US gallons. In 2017, the biofuel production in the USA amounted to approximately 36.9 million metric tons of oil equivalent and in Brazil it amounted to 18.4 million metric tons of oil equivalent. The largest consumer of biodiesel is the US army. According to the US Energy Information Administration, the US production of biodiesel was 131 million gallons in February 2019. The US Department of Energy predicts that the use of foreign petroleum, which currently feeds 56 % of US's demand, will grow to 68 % by 2025. Consequently, a serious interest in alternative energy sources is now being fostered for reducing dependence on non-renewable foreign energy sources.

2.8 Conclusion and Future Perspectives

Biofuels are considered to be the main potential replacement for fossil fuels in near future. Owing to ever increasing prices and shortage of fossil fuels, significant progress has been made in the last several years in the area of biofuel production. Aquatic weed biomass is, so far, the most economical and globally highly available. Nowadays, priority is given towards utilizing aquatic weed biomass for biofuel production. The merits of utilizing aquatic weeds for biofuel production include: (i) they do not require arable land that could be used for crops or forests; (ii) their

superiority in adaptability and productivity; (iii) their ability to accumulate sugars and starch; and (iv) aggressive growth characteristics and exceptional regeneration features. However, aquatic weeds cause negative ecological and economical impacts and require huge monetary expenditure to eradicate the weeds. Utilizing the weed biomass for converting too biofuel or other forms of energy would greatly help to offset this cost and would also be beneficial to the local ecosystem. Gene technologies can play a key role in enhancing the efficiency of weed biomass conversion to biofuels. Genetic underpinning such as cell wall chemistry, stem thickness, branching habit, competition for light and growth rates, will maximize biomass yield per unit land area. Different pretreatment techniques have been explored to convert weed biomass into bioethanol. However, current technologies are still inadequate for bioethanol production from weed biomass to compete with starch and sugar based bioethanol in terms of production yield and cost. Further research to establish cost effective and efficient conversion processes including pretreatment technique(s) for a wide range of weed biomass is needed. Computer-based predictive models can help in the selection, design, optimization, and process control of pretreatment technologies and subsequent conversion of pretreated weed biomass to biofuel. There is a need to optimize processes for microbial fuel cells, feedstock production, biomass pretreatment, enzyme hydrolysis, genetic manipulation of microbial cells, and their application in the biofuel industry, bioreactor systems, and economical processing technologies for biofuel residues. There is also a need to train researchers and scientists across diverse disciplines and industrial sectors in which biofuel technologies and related research and experimentation are pursued. With strong support from various governments, bioethanol production from weed biomass will play a major role in meeting energy demand globally. Though several aquatic weeds have biofuel potentiality, economical and industrial feasibilities of aquatic weed-based biofuel production should be considered in further researches in order to practical implementation.

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Chapter 3
Aquatic Weeds: A Potential Pollutant Removing Agent from Wastewater and Polluted Soil and Valuable Biofuel Feedstock

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3.1 Introduction

The worldwide growing population needs water security for communities will rise higher in the near future. Natural freshwater sources globally decreasing rapidly; therefore, it is expected that clean water will be secured sincerely for basic human needs (Rezania et al. 2016c). Due to rapid urbanization and industrialization, wastewater discharge into the environment increasing day by day. In the last few years, developed countries are updating the environmental policies and dedicated to reducing water pollution as well as developing an efficient and self-sustainable approach for wastewater treatment. For higher efficacy particularly in metropolitan cities, the advancement of recognized traditional treatment methods of water and wastewater is in a need to be further developed. Therefore, strict regulations should

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be implemented to discharge of wastewater in rivers and ponds, which should be decided based on wastewater quality and its sources such as household wastewater or industrial wastewater (Rezania et al. 2016c). Various regulations had been adopted by the governments that controlled industrial action regarding the discharge of heavy metal contaminated wastewater into the environment (Sud et al. 2008). However, wastewater is characterized as municipal wastewater and industrial wastewater sources (Rezania et al. 2016c, 2015a). Municipal wastewater contains more organics, inorganic, turbidity, and suspended solids, while industrial wastewater contains heavy metals, cyanide, toxic organics, nitrogen, phosphorous, phenols, and color (Rezania et al. 2015b). These contaminants cause several adverse effects on the environmental and also raise economic issues (Chan et al. 2009). Most ecological contaminants have negative impacts on the quality of soil and water, animal nutrition, in addition to human health, which decrease the economic growth of the contaminated regions (Jomova and Valko 2011). Nowadays, the removal of soil heavy metal contamination is of utmost importance owing to its high vulnerability.

In recent years, researchers are developing effective bioremediation methods because of the crucial problem of heavy metals present in wastewater, which contaminates groundwater. Nowadays, several methods are being explored to identify cost-effective and more efficient technologies, to alleviate the quantity of wastewater and simultaneously enhance the effluent quality (Barakat 2011). However, commonly used methods of wastewater remediation like flocculation, oxidation, chemical precipitation, adsorption, coagulation, membrane filtration, ion-exchange, ozone/H_2O_2, photocatalytic degradation, and electrochemical methods are few physicochemical approaches that are being widely studied. However such techniques have been considered non-eco-friendly and expensive (Martín-Lara et al. 2014; Olguín and Sánchez-Galván 2012; Rezania et al. 2016c, 2015a). In the last few years, several investigations showed that heavy metal removal could be effective by physical, chemical, and biological treatment methods (Fu and Wang 2011). Mainly physical and chemical methods demand high operating costs therefore such methods are not eco-friendly. Thus, the research interest has been shifted toward phytoremediation, which is a cost-effective and eco-friendly tool and could be an appropriate approach for heavy metal remediation from wastewater as well as for improving the air quality (Ladislas et al. 2012). Heavy metals could be removed from contaminated soils or wastewater using different biological processes such as phytoremediation/bioremediation, even though this is a challenging issue due to undegradable property of heavy metals and persist in the soil (Jomova and Valko 2011; Rezania et al. 2016c, 2015b). For bioremediation, several microorganisms such as bacteria, fungi, yeast, and microalgae could be employed in the contaminated regions for heavy metal remediation (Ojuederie and Babalola 2017). However, microbial bioremediation is not really viable and effective at a large scale due to the high cost of microbial inoculums and unfavorable growth conditions at contaminated sites (Verma and Kuila 2019). To overcome, several important issues of microbial bioremediation technology, plant-based remediation (phytoremediation) can be a viable method for heavy metals removal from the contaminated area

(Ojuederie and Babalola 2017). Phytoremediation works against the removal of polluting organic materials and heavy metal form contaminated sites by different plant species. Phytoremediation is a cost-effective, eco-friendly, and effective approach to restore any polluted environment particularly that of heavy metals, and reduce air pollution (Ojuederie and Babalola 2017). Several aquatic floras like water hyacinth, Salvinia, water lettuce, Eurasian water milfoil, Typha, common coontail could be used for wastewater treatment. Many researchers have reported that these plants show great potential to remove a broad range of pollutants, including chemical oxygen demands (COD), heavy metals, total suspended solids (TSS), dissolved solids (DS), total nitrogen (TN), and total phosphorous (TP) contents (Fu and Wang 2011; Ladislas et al. 2012; Lu et al. 2011, 2010; Martín-Lara et al. 2014; Rezania et al. 2016c; Sindhu et al. 2017). Furthermore, the biomass of aquatic plants could be used for biofuel production (Kaur et al. 2018).

This chapter elaborates on the negative influence of various potential pollutants to our surrounding ecosystems and the role of model aquatic weed "water lettuce (*Pistia stratiotes*)" for its effective remediation ability for these pollutants. Moreover, the possible prospects of harvested water hyacinth (*Eichhornia crassipes*) and water lettuce (*P. stratiotes*) biomass after phytoremediation for biofuel production are also discussed.

3.2 Aquatic Weeds and Its Characteristics

Several aquatic weeds including water hyacinth used as ornamental plants because of their sticking appearance to mankind for centuries (Rezania et al. 2015a). These aquatic weeds have long roots that are usually suspended in water. Such structure of water hyacinth roots may provide an appropriate niche for the aerobic microbes to act in the sewage. A normal water hyacinth consists of moisture (95.5%), N (0.04%), ash (1.0%), P_2O_5 (0.06%), K_2O (0.20%), and other organic matters (3.5%). While, on zero-moisture basis, it contains more organic matter (about 75.8%), ash (24.2%) and nitrogen (1.5%) out of this about 28.7% is pertaining to K_2O, 1.8% for Na_2O, 12.8% CaO, 21.0% Cl, and 7.0% P_2O_5. The crude protein contents were assessed (crude protein ¼ amount of nitrogen 6.25) using Kjeldahl method which revealed the presence of methionine, phenylalanine, threonine, glycine, isoleucine, valine, and leucine equal to 0.72, 4.72, 4.32, 5.34, 4.32, 0.27, and 7.20 g, respectively (Malik 2007). Several other weeds, for example, *Salvinia molesta*, *P. stratiotes*, *Myriophyllum spicatum*, *Typha latifolia*, and coontail (*Ceratophyllum demersum*) are available worldwide as they can grow in broad temperature zones as reported in Table 3.1 and be used for phytoremediation of polluted waters and soils. Among the aquatic weeds, water hyacinth (*E. crassipes*) and water lettuce (*P. stratiotes*) are free-floating perennial aquatic plant native to tropical and subtropical South America, and nowadays widespread in all tropic climates. However, its faster growth rate, high tolerance to pollution, and quick absorption capacity of heavy metal and nutrient qualify it for use in wastewater

Table 3.1 Global spread of aquatic weeds in different zones of the world. Adapted from Kaur et al. (2018)

Type of weeds	Weed species	Distribution
Free-floating	*Eichhornia crassipes*	Nearly all tropical and subtropical zones
	Pistia stratiotes	Nearly all tropical and subtropical zones
	Salvinia molesta	Australia, Asia, United Kingdom, New Zealand, and parts of America
	Azolla spp.	Nearly all Tropical and subtropical zones
	Lemna spp.	Africa, Asia, Europe, and USA
	Ipomoea aquatica	Nearly all tropical and subtropical zones
Submerged	*Hydrilla verticillata*	Asia, Europe, United Kingdom, Africa, and Australia
	Vallisneria spirallis	Iceland, New Zealand, Asia, Africa, and Europe
	Potamogeton sp.	Asia, Africa, the Middle East, Australia, and Europe
	Myriophyllum spicatum	All continents except Australia and Antarctica
	Elodea canadensis	Australia, Asia, Africa, Europe, New Zealand, and parts of America
Emergent	*Typha* sp.	Tropical, subtropical, temperate, and coastal zones
	Phragmites australis	All continents except Antarctica
	Ipomea carnea	Asia, Africa, and USA
	Alternanthera philoxeroides	Australia, China, New Zealand, Sri Lanka, Thailand, and the USA

treatment (Rahman and Hasegawa 2011). The pollutant removal efficiency of water hyacinth is higher due to its faster growth rate and favorable climatic conditions, while other aquatic weeds have lower pollutant removal efficiency due to lower growth rate and lower tolerance limit of heavy metals as compare to water hyacinth. Therefore, water hyacinth represents a reliable alternative for heavy metal bioremediation in the aquatic system and the use of water hyacinth in phytoremediation technology should be considered (Rahman and Hasegawa 2011; Rezania et al. 2016a, 2016c, 2015b).

3.3 Application of Aquatic Weeds

A literature study showed that aquatic weeds have been employed for several applications such as the remediation of heavy metals from the aquatic environment using *E. crassipes*, *Lemna minor*, and *P. stratiotes* (Hua et al. 2012; Rezania et al. 2016c). Figure 3.1 shows a few such flora that are prevailing in the phytoremediation process. Several review articles were published in the past five years and

Water Hyacinth (*Eichhornia crassipes)*

Water Lettuce (*P. statiotes*)

Duckweed (*Lemna* sp.)

Azolla (*Azolla filiculoides)*

Cattail (*Typha latifolia*)

Giant Salvinia *(S. molesta)*

Fig. 3.1 Most commonly available aquatic weeds with higher cellulose, and hemicellulose content

reported that free-floating aquatic plants could be an eco-friendly choice for heavy metals removal from wastewater or aqueous environment.

Aquatic weeds help in the removal of various inorganic forms of heavy metals present in the contaminated water bodies, for instance, arsenic can exist in inorganic forms (As(V) and As(III)) as well as in methylated forms (MMAA(V)) and DMAA (V)) in the aqueous environment (Rahman and Hasegawa 2011). The uptake mechanism of Arsenic by terrestrial hyper accumulating plants has been widely studied (Rahman and Hasegawa 2011; Tripathi et al. 2007; Zhang et al. 2009; Zhao et al. 2009). Three main systems were proposed for arsenic uptake by aquatic weeds: (i) phosphate transporters based active-uptake; (ii) aquaglyceroporins based passive uptake; and (iii) direct adsorption by the surface of roots. For active transport through phosphate uptake transporters As(V) and phosphate (chemical

analogs) compete for uptake carriers in the cytoplasmic membrane. Therefore, more As(V) is expected to be desorbed in the solution with the increase in phosphate concentration (Mkandawire et al. 2004; Smith and Read 2008). A detailed mechanism for uptake of As(V) through phosphate transporters in aquatic plants discussed by Tripathi et al. (2007). On the other hand, passive uptake of As(III) and methylarsenicals through aquaporins/aquaglyceroporins in plants has not yet been identified. Moreover, physicochemical adsorption on the root surface was proposed to be an alternative mechanism for As(V) accumulation in aquatic plants (Robinson et al. 2006). Here, suspended iron oxides (Fe-plaque) on the aquatic plant surfaces adsorb and accumulate Arsenic. A positive correlation was observed between arsenic and iron concentrations in aquatic plants (Robinson et al. 2006). Furthermore, after the uptake of arsenic by aquatic plants, its metabolism and detoxification could be carried out by various routes such as reduction of As(V) to As(III) is mediated by groups of peptides such as glutathione (GSH) and by similar biocatalysts (Bleeker et al. 2006; Rahman and Hasegawa 2011; Zhao et al. 2009).

After the heavy metal removal, aquatic weeds biomass could be used different application. Aquatic weeds contain fibrous tissue besides having high protein and water contents. Due to aquatic biomass properties, which could be utilized as feed to produce biofuel like biogas, biohydrogen, ethanol production (Barua and Kalamdhad 2018; Chan et al. 2009; Kaur et al. 2018; Malik 2007; Rezania et al. 2016a, 2015a). Moreover, after biofuel production, the residual biomass could use

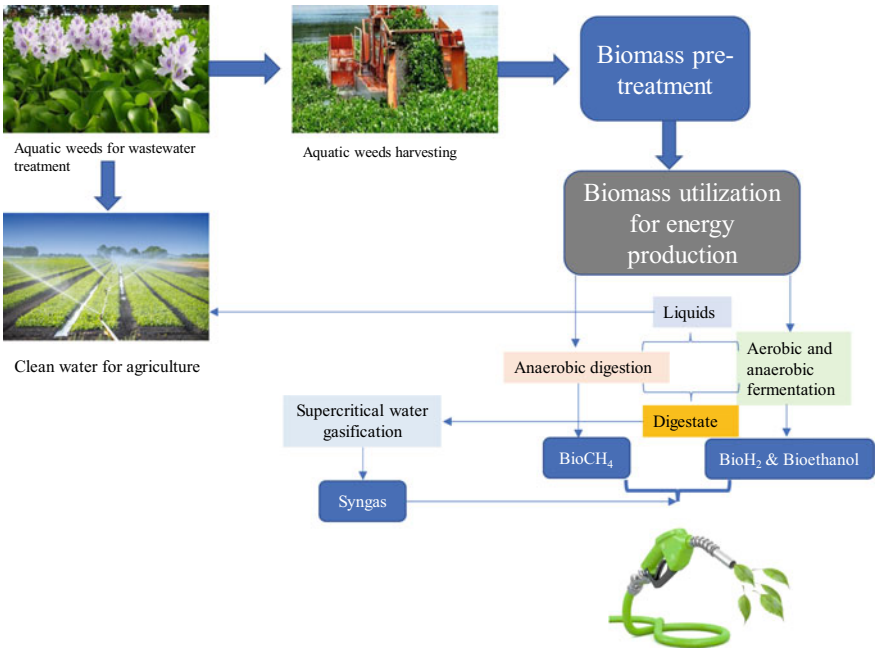

Fig. 3.2 Systematic application of aquatic weeds for different biofuel production

supercritical water gasification (Mehariya et al. 2018a; Molino et al. 2016; Panepinto et al. 2016; Siciliano et al. 2018). The detailed use and application of aquatic weeds are discussed in the next section and systematic representation of different application showed in Fig. 3.2.

3.4 Applications of Aquatic Weeds in the Removal of Pollutants from Wastewater

Wastewaters contain water with a huge amount of inorganic and organic chemicals along with heavy metals that could be generated from common households, industries, and other commercial activity (Dixit et al. 2011). The wastewater contaminates groundwater as well as rivers, ponds, etc., therefore, it should undergo proper treatments before its final discharge. Therefore, the aquatic plant could useful to absorbent these pollutants from wastewater. The aquatic weed bearing elongated roots assist in the absorption of pollutants present in the wastewater, mainly the heavy metals consequently it helps in recovering the water quality (Sooknah and Wilkie 2004). Water hyacinth can remove Cu with higher removal efficiency, while lower removal efficiency of Cd was observed from wastewater (Liao and Chang 2004). Gupta and Balomajumder (2015) reported about the potential of water hyacinth to efficiently remove phenol (up to 99%). The heavy metals absorbed through the roots of the aquatic weed gets translocated into the stems and other tissues (Jadia and Fulekar 2009). Table 3.2 showed the recent reports conducted for the heavy metal removal by aquatic plants.

Moreover, aquatic weed such as water hyacinth is well known and worked at small laboratory-scale setup to pilot as well as large scale to remove organics from wastewater (Barakat 2011; Putra et al. 2015; Rahman and Hasegawa 2011; Rezania et al. 2016a, 2016c, 2015b). Similarly, other fields and laboratory-scale works have demonstrated the efficacy of water hyacinth to remove a plethora of inorganic and organic compounds found in the swine wastewater (Valero et al. 2009). Water hyacinth removed 64.4% of COD, 21.8% TN, and 23.0% TP from duck farm wastewater. The transparency of wastewater and its DO both were significantly improved the former case and achieved upto 2.5-folds higher in comparison to untreated wastewater (Jianbo et al. 2008). Moreover, a mixture of hyacinth and duckweed was found to reduce 79.0% of TN and 69.0% of TP from dairy wastewater (Tripathi and Upadhyay 2003). On the other hand, Ismail et al. (2015) worked on another mixture of aquatic plants (a combination of water hyacinth and water lettuce) and found efficient uptake of nitrate, orthophosphate, nitrite, and ammoniacal nitrogen from domestic wastewater. Water hyacinth was found to display better nitrate reduction efficiency as compared to orthophosphates (Ismail et al. 2015). Several studies showed that aquatic plants could remove inorganic and organic pollutants from the wastewater as summarized in Table 3.3.

Table 3.2 Heavy metal removal capacity of aquatic weeds. Adapted from Kaur et al. (2018)

Aquatic weeds	Heavy metals/ nutrients	Source	Adsorption capacity	References
Eichhornia crassipes	Cd(II), Pb(II)	Aqueous solution	75% for Cd and 90% for Pb	Ibrahim et al. (2012)
	Fe(II and III)	Fe-rich wastewaters. in constructed wetlands	6707 Fe mg/kg dry weight	Jayaweera et al. (2008)
	Cu(II), Cd(II), Pb(II), and Zn (II)	Anaerobic up flow packed bed reactor with water hyacinth ponds	98% for Cd, 99% for Cu, 98% for Pb, and 84% for Zn	Sekomo et al. (2012)
Lemna sp.	Se(IV and VI)	Selenium-Rich Mining Wastewater	30.05% Se	Miranda et al. (2014)
	Cr(III), Ni(II), Cd(II) and Pb (II)	Aqueous solution	Cr-98%, Ni-57%, Cd-88%, and Pb-86% at 2 ppm, 2 ppm, 0.5 ppm, and 8 ppm treatments	Lokuge (2016)
Azolla sp.	Cu(II) and Cr (III)	Aqueous solution	Cu-6%, Cr-62.5%	Pandharipande and Gadpayle (2016)
	Ni(II), Cr(VI)	Aqueous solution	Ni-28443 µg/g, Cr-12383 µg/g	Sood et al. (2012)
Pistia stratiotes	Cr(III) and Co (II)	Aqueous solution	Cr-100%, Co-86	Prajapati et al. (2012)
Salvinia molesta	Hg(II), Pb(II)	Aqueous solution	Hg-74%, Pb-85% at 100 mg/L	Kumari et al. (2017)

3.5 Application of Aquatic Plants for Biofuel Production

Aquatic weeds are naturally grown flora with a faster growth rate, they provide a vast supply of cheap feedstock for biofuel production. The prime motive to utilize these noxious weeds besides regulating their excessive growth is that they show significantly higher productivity rates than terrestrial bioenergy feedstock crops (Miranda et al. 2016). For instance, the annual yield of water hyacinth is 100 dry tons/ha in natural lakes while the highest yield obtained for switchgrass in the USA is 25 dry tons/ha (Kaur et al. 2019; Hronich et al. 2008; Wullschleger et al. 2010). However, aquatic weeds contain a high amount of cellulose, hemicellulose, while low lignin content as compared to lignocellulosic biomass as shown in Table 3.4. These compounds are simply hydrolysed into fermentable sugars, which further

Table 3.3 Recent studies for the removal of organic and inorganic using water hyacinth. Adapted from Rezania et al. (2015b)

Type of waste water	Removal of organic and inorganic pollutants	Key improvements after remediation by water hyacinths and other aquatic weeds	References
Dye wastewater	Nitrogen, ammonium nitrogen, (BOD), pH, hardness, (TDS), (DO), conductivity, (COD), nitrate	The experiment was carried using 25%, 50%, 75%, and 100% of waste water. A significant decrease in all the parameters was noticed. Water hyacinth showed better efficiency with 25%–50% of waste water	Shah et al. (2010)
Eutrophic lake	Transparency, (TN), (NH_4), (NO_3), (TP), (PO_4), (COD)	Water quality improved surrounding the water hyacinth mats, also in most of the parameters the concentration was found to be decreased	Wang et al. (2018)
Domestic waste water	(COD), (TN), (TP)	80% of (COD), 75% of (TN) and 75% of (TP) reduction happened during the first week of experiment. It was found that 20% or 15 L of water reduction occurred weekly and 40% increase in the plant biomass was observed after 14 days	Rezania et al. (2013)
Polluted river water	(TDS), total hardness, sulfate, phosphate, (EC), pH, (NO_2), (NO_3), (TN)	Significant reduction of electrical conductivity (25% decrease), total dissolved solids (TDS) (26%), sulfate (45%), phosphate (33%) and total hardness (37%) between the sample points SR1 and SR3 were obtained	Moyo et al. (2013)
Municipal waste water	(BOD), (COD), (NO^{3-}–N) (TKN), (PO_4^{3-}–P)	Removal of parameters for mixed culture of *Eichhornia crassipes* and *Salvinia natans*: 84.5% of (BOD) 83.2% of (COD) 26.6% of (NO^{3-}–N) 53.0% of (TKN) 56.6% of (PO_4^{3-}–P)	Kumari and Tripathi (2014)

(continued)

Table 3.3 (continued)

Type of waste water	Removal of organic and inorganic pollutants	Key improvements after remediation by water hyacinths and other aquatic weeds	References
Domestic waste water	pH, (COD), (PO_4^{3-}), (NO_3), (NH_3), (TOC), biomass growth rate	Optimum removal rate for all the parameters was found to be between 12- and 15-days using water hyacinth Optimum growth rate was found in 18 days with removal rate of (COD) 95%, TOC 45%, (PO_4^{3-}) 45%, (NH_3) 85%	Rezania et al. (2016b)
Domestic waste water	(TSS), (COD), (NH_4^+), (PO_4^{3-})	Comparison of Water hyacinth and Water morning glory showed: 37.8%–53.3 for TSS; 44.4–53.4% for COD; 56.7–61.4% for PO_4^{3-} and 26.8–32.6% for NH_4^+. Lower values belong to Water morning glory and higher values belong to water hyacinth	Loan et al. (2014)
Domestic sewage water	Nitrate, phosphate, ammonia	Water hyacinth + papaya stem: 67% ammonia reduction, 74% nitrate, and 71% phosphate removal	Anandha Varun and Kalpana (2015)
Domestic wastewater	COD, BOD, TN, TP, TSS, PO_4–P, NH_3–N	COD reduction: (79%), BOD removal: (86%), TN: (76.61%), TP: (44.84%), TSS: (73.02%), PO_4^{3-}–P: (38.69%), NH_3–N: (72.48%) at HRT of 14 h was achieved	Valipour et al. (2015)

biologically transformed into biofuels (Mishima et al. 2008). Therefore, aquatic weed biomass has several advantages and considered as a potential feedstock for biofuel production. Especially, due to lower lignin contents, pre-treatment of aquatic weed biomass is faster and process operation is easy for aerobic and anaerobic fermentations. Moreover, aquatic weeds have a greater amount of protein and fat as compared to agricultural wastes, which can be easily converted into $BioH_2$ and $BioCH_4$ (Kaur et al. 2018). $BioH_2$ and $BioCH_4$ could be used for generating energy, which has higher efficiency and could be used for transportation fuels (Karthikeyan et al. 2018, 2017; Kumar et al. 2015b, 2015a, 2014; Mehariya et al. 2018b; Patel et al. 2014). Despite their unique phytoremediation properties, the heavy metal contents within the biomass did not influence the fermentation process to a large extent (Jain et al. 1992). Several studies had been conducted in the past in order to assess the influence of heavy metals present in such biomass on

anaerobic digestion (AD) or biofuel production. Heavy metals are usually considered to be the inhibitors of AD; however, it was argued that the threshold levels of these metals required for the inhibition never reaches in the biomass (Maneein et al. 2018). In contrast, when heavy metals were removed from the biomass hydrolysate using cryogel adsorbents, there was a reduction in the biogas yield. This was possibly due to high sulfate levels after heavy metal removal that led to more H_2S generation. Therefore, the presence of heavy metals to some extent indeed helps the fermentation process; however, their levels need to be regulated for a large-scale fermentation setup. Mishima et al. (2008) used aquatic weeds (water hyacinth and water lettuce) for ethanol production and found that 140–170 mg/g dry weight of water hyacinth and 150–160 mg/g dry weight of water lettuce. In another study, the potential of aquatic weed biomass was explored using a microreactor-based trial for bioethanol production and data showed that 218, 197, 215, and 189 mg ethanol in per g dry biomass could be attained from *Lemna gibba*, *L. minor*, *P. stratiotes*, and *Eichhornia* sp., biomass respectively. Therefore, aquatic weeds biomass could be considered as useful feedstock for liquid (ethanol, butanol, biodiesel, etc.) biofuel production, which could be further used as a transportation fuel. Moreover, bioethanol productivity could be further increased by optimizing the production strategies (Gusain and Suthar 2017). Kaur et al. (2019) used aquatic weed biomass for bioethanol production and compared the potential of different biomass as feedstock for bioethanol production. The result showed that maximum bioethanol production could be attained using *E. crassipes* biomass (231 mg/g) followed by *L. minor* biomass (185 mg/g) and *Azolla microphylla* biomass (167 mg/g), which could be possible due to higher cellulose content in *E. crassipes* biomass as compared to other used aquatic weed biomass.

Chuang et al. (2011) used water hyacinth biomass for $BioH_2$ and $BioCH_4$ production. Results showed that maximum $BioH_2$ production (38.2 mmol $BioH_2$/L/d) and $BioCH_4$ production (29.0 mmol $BioCH_4$/L/d) were obtained using water hyacinth biomass with a concentration of 40 and 80 g/L, respectively. Güngören Madenoğlu et al. (2019) use water lettuce biomass for biogas production and found 321 ml/gVS biogas with high methane content during the AD of 30 g dry biomass at 35 °C. Recently, Kaur et al. (2019) used different aquatic weeds biomass for the simultaneous production of bioethanol and $BioCH_4$ using *E. crassipes*, *L. minor*, and *A. microphylla* biomass. The study showed that higher glucose production (36.5–44.2 g/L) and ethanol yield (167–231 mg/g dry biomass) could be achieved using different aquatic weeds. Moreover, AD of aquatic weeds prior to ethanolic fermentation produced a relatively higher $BioCH_4$ yield (32.9–52.5 m^3/ton) (Kaur et al. 2019). The potential of different aquatic weeds biomass as feedstock for biofuels such as bioethanol, $BioH_2$, and $BioCH_4$ production is shown in Table 3.5.

Table 3.4 Biochemical composition of different biomass. Adapted from Kaur et al. (2018)

Biomass		Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
Aquatic weed biomass	*Eichhornia crassipes* (Water Hyacinth)	24.5	34.1	8.6	Ruan et al. (2016)
	Pistia statiotes (Water Lettuce)	27.5	29.7	3.5	Sivasankari and David (2016)
	Azolla filiculoides (Azolla)	21.8	13.5	10.3	Miranda et al. (2016)
	Lemna sp. (Duckweed)	10.0–24.5	3.5	3.1	Xu and Deshusses (2015)
	Salvinia molesta (Giant Salvinia)	32.0	26.0	13.7	Sciessere et al. (2011)
	Typha latifolia (Cattail)	38.5	37.0	12.8	Sopajarn and Sangwichien (2015)
Energy crops	Switchgrass (*Panicum virgatum*)	45.0	31.4	12.0	Bajpai (2016)
	Pine (*softwood tree*)	45.0–50.0	25.0–35.0	25.0–35.0	
	Other grasses	25.0–40.0	35.0–50.0	10.0–29.9	
	Silvergrass (*Miscanthus giganteus*)	37.0–45.0	19.0–25.0	17.0–21.0	Haffner et al. (2013)
Agricultural residues	Wheat straw	38.4	19.7	16.9	Rajan and Carrier (2014)
	Rice straw	41.7	18.3	16.6	Zheng et al. (2013)
	Corn stover	37.0	31.3	17.8	Saha et al. (2013)
	Sugarcane bagasse	34.0	27.0	18.0	Binod et al. (2012)

3.6 Key Challenges Using Aquatic Weeds for Wastewater Treatment and as Feedstock for Biofuel Production

Several studies have been reported that controlling the growth of aquatic weed is quite difficult. After the wastewater treatment process, each aquatic plant need to harvest before the discharge of treated wastewater because a single plant can outgrow severely in the entire ecosystem and contaminate a water source. Especially, water hyacinth weed grows rapidly and found to be difficult to eradicate from the water bodies (Rezania et al. 2015b). Therefore, proper eradication should be

Table 3.5 Bioenergy production potential of aquatic weeds

Biofuels	Aquatic weeds	Productivity	References
Bioethanol	*Eichhornia crassipes*	231 mg/g	Kaur et al. (2019)
	Lemna minor	185 mg/g	
	E. crassipes	189 mg/g	Gusain and Suthar (2017)
	L. minor	218 mg/g	
	Lemna gibba,	197 mg/g	
	Pistia stratiotes	215 mg/g	
	E. crassipes	0.224 mg/g	Masami et al. (2008)
	Lemna sp.	12 mg/g	Lee et al. (2016)
$BioH_2$	*E. crassipes*	751.5 ml/g VS	Cheng et al. (2013)
	E. crassipes	13.65 ml/g VS	Lay et al. (2013)
	E. crassipes	596.1 ml/g VS	Su et al. (2010)
	E. crassipes	51.7 ml/g VS	Cheng et al. (2010)
	Lemna sp.	75 ml H_2/g	Xu and Deshusses (2015)
$BioCH_4$	*L. minor*	52.5 m^3/ton	Kaur et al. (2019)
	Azolla microphylla	32.9 m^3/ton	
	E. crassipes	40.6 m^3/ton	
	E. crassipes	552 L/kg VS	Mathew et al. (2015)
	Salvinia sp.	221 L/kg VS	
	E. crassipes	~133 L/kg^{-1} VS	O'Sullivan et al. (2010)
	Cabomba Caroliniana	~110 L/kg VS	
	Salvinia molesta	~77 L/kg VS	
	E. crassipes	143.4 ml/g VS	Cheng et al. (2010)
	Typha sp.	104 ml/g VS	Nkemka et al. (2015)

considered before the discharge of treated water in water bodies. Moreover, after aquatic weed harvesting, it could be used as feedstock for bioenergy production. However, the use of aquatic biomass as feedstock for biofuel production faces several challenges that demand effective and quick process due to high moisture content. The wet biomass needs to be dehydrated quickly or need to transport to biofuel production units due to higher degradability due to moisture content. Therefore, these are the main challenges for biofuel production. Integration of wastewater treatment and aquatic weed cultivation for biofuel production, as a centralized treatment model needs to be developed, where biomass could be used biofuel production to reduce the overall economics (Kaur et al. 2018).

3.7 Conclusion and Future Prospective

This book chapter highlighted the potential of aquatic weeds for wastewater treatment and further the biomass could consider as potential feedstock for biofuel production. In recent years, the research interests have increased for heavy metal removal using the aquatic weeds due to its several advantages. However, the several parameters influence the removal efficiency due to lower growth rate, lower tolerance limit of heavy metals. Therefore, genetic engineering approach could help to develop genetically modified aquatic plants with higher growth rate, higher tolerance, and removal rate of different heavy metals and need to be further investigated. Moreover, in the future, using biotechnological approach through specific metal gene identification of hyper accumulators is vastly suggested. The environmental conditions and wastewater characteristics need to be considered. For biofuel production, using aquatic weeds as potential feedstock, it demands extensive research to enhance the economic viability and feasibility for biofuel production. Therefore, coupled wastewater treatment and bioenergy production could be a good approach. Overall, several benefits need to consider for development of aquatic weeds for wastewater treatment and biomass could be used as possible feedstock for biofuel production.

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Chapter 4
Parthenium hysterophorus: Weed to Value

Supriya Ratnaparkhe and Milind B. Ratnaparkhe

4.1 Introduction

Parthenium hysterophorus L. is one of the most invasive weeds and belongs to the family Asteracaeae. The weed which is commonly called carrot grass, Santa Maria, Santa Maria feverfew or famine weed is native to American tropics but has spread rapidly to over thirty countries across Africa, Asia and Australia (Fig. 4.1). *Parthenium* is said to have been introduced into Asia and Africa from America during the 1950s with shipments of cereal and grass seed. It exhibits continuous and profuse flowering throughout its life cycle which ranges from 4 to 6 weeks. Due to the continuous flowering and the high adaptability of the plant, the seed productivity is also very high. The seeds are lightweight with strong regenerative capability and remain dormant in adverse conditions (Dagar et al. 1976). The growth of this weed is supported by a wide range of environmental conditions and hence in such diverse habitats. It spreads easily through contaminated grains, farm machinery and crop products. Although it grows luxuriously throughout the year, its germination, flowering and seed setting are significantly affected by environmental conditions due to which it exhibits different stages of life cycle and sometimes shows up to three generations in one year (Tamado et al. 2001; Kushwaha and Maurya 2012). The habitat of this *Parthenium* ranges from natural to agro-ecosystem, although the incidence is higher in wastelands, road sides, railway tracks and rock crevices (Kumar and Rohatgi 1999). It establishes more aggressively in bare lands after droughts and floods, as compared to extensive vegetation or pastures in undisturbed land. However, its invasion is a serious threat to conservation and sustainability of biodiversity in any given region.

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Fig. 4.1 Distribution of *Parthenium* weed across various countries of the world Adapted from Shabbir et al. (2011)

Parthenium weed not only causes huge loss to crop production and plant biodiversity owing to its aggressive dominance, but also has adverse effect on the health of humans and livestock. It is known to cause allergic respiratory problems, contact dermatitis, mutagenicity, allergic rhinitis, breathlessness and even diarrhoea in mammals, depending upon the climatic conditions. The weed causes allergy and is unpalatable to grazers.

4.1.1 Morphology

Morphologically, just like other members of Compositae family, the plant has a typical flower head called the capitulum which is cluster of many tiny florets. This capitulum is surrounded by an involucre of bracts. The plant has two stages in its life cycle, namely (1) juvenile or rosette stage with a carpet of large leaves at the bottom and smaller, simple pinnatisect leaves on the top; (2) the adult stage is procumbent with highly branched herb that has tough and woody stems (Fig. 4.2). Each plant produces millions of pollens and thousands of seeds. The pollens are wind pollinated. The plant grows in a wide range of temperature, light conditions and soil conditions except saline soils or seashore as the salinity is not conducive to its flowering.

Fig. 4.2 Morphological characteristics of Parthenium hysterophorus **a** Juvenile stage **b** Adult stage **c** Inflorescence Photo credit: Dr. D.N. Baraskar

4.2 Related Species

Many species of the genus *Parthenium* have common flavonoids. On the basis of the presence of kaempferol and quercetin 3-0-glycosides *P. hysterophorus*, *Philodendron bipinnatijidum* and *Pythium glomeratum* were found to be closely related, while the North–South American species pair *P. glomeratum* (Argentina) and *P. bipinnatifidum* (Mexico) yielded quercetagetin 3,7,3′-trimethyl ether as the major aglycone (Shen et al. 1976). Several of the members of this genus are known to cause allergic reactions in humans and livestock. A related species *Parthenium argentatum*, also known as guayule, is commonly used for rubber making. In an experiment involving interspecific crosses between members belonging to the genus *Parthenium*, namely *P. argentatum, P. stramonium, P. iomeniosum, P. incanum, P. tomentosum* and *P. hysterophorus,* it was found *P. hysterophorus* is too remote in its relationship to the other species of *Parthenium* with a chromosome number $2n = 34$ (Rollins 1946).

4.2.1 *Genetic Diversity Studies in* P. hysterophorus

The main purpose of understanding the patters of genetic structure is to understand the invasion history and levels of gene flow amongst populations. Very limited information exists regarding the genetic diversity in *P. hysterophorus* at molecular level. In a study of genetic structure of 95 samples collected from across Pakistan and Australia, using inter simple sequence repeat (ISSR) markers, it was found that the genetic diversity ranged between 0.193 and 0.278. Although the genetic diversity within the population was high, a limited gene flow amongst the population was observed. The results showed that the *Parthenium* weed in Pakistan is genetically heterogeneous and may have been the result of multiple introductions (Jableen et al. 2015). To detect the genetic diversity within *Parthenium* species, other molecular markers such as microsatellites and chloroplast sequences have also been used. Microsatellite markers that can differentiate closely related species and can detect the diversity within species have been used to genetically characterize *P. hysterophorus.* Qian et al. (2012) demonstrated the utility of 15 polymorphic microsatellite markers that were specifically characterized for *P. hysterophorus*, in genetic diversity studies and eventually understanding the invasion history of this species globally. Chloroplast sequencing can be very helpful in identification of species when a particular sequence is used as a DNA tag or barcode. A complete chloroplast genome sequencing of a related species *P. argentatum* was carried out and subjected to comparative analysis to identify DNA barcodes to differentiate *Parthenium* species and lines. The research showed that using the matK and psbA-trnH spacer chloroplast DNA barcodes, *P. hysterophorus* can be differentiated from *P. argentatum* (Kumar et al. 2009).

In China, *P. hysterophorus* is mostly found in Southern parts of the country; however, isolated populations were reported from Shandong which is a coastal province of the People's Republic of China. An investigation of the genetic structure of 18 invading populations using a combination of inter simple sequence repeat and chloroplast DNA sequence markers (*trnQ-5'rps16*) examining the relationship of Shandong and southern populations in China revealed that Shandong population is genetically different and has apparently higher levels of genetic diversity than most populations in southern China (Tang et al. 2009). Another study was carried out in Australia where the researchers used samples collected from its introduced range in Australia (including two distinct biotypes) and the native ranges in Costa Rica, Mexico and the United States of America. The aim of this study was to identify the informative gene regions and sequences from the various samples of the weed. Five gene regions were investigated including one nuclear region (ITS) and four chloroplast regions (trnH-psbA, trnL intron, trnL-trnF spacer and matK). However, not much genetic diversity was found in any of the samples, and it was concluded that the regions selected for the study were not optimal for evaluation of the genetic diversity in *Parthenium* weed due to the lack

of polymorphism or due to extremely limited genetic diversity. Other high throughput techniques could prove to be more informative as far as population genetic structure of *Parthenium* weed is concerned.

4.3 Harmful Effects of *Parthenium* Weed

Parthenium is one of the worst weeds mainly because of its invasiveness and high spreading capacity, and due to its adverse impact on humans and livestock health. Humans that get exposed to the plants or pollens can have severe allergic reactions such as dermatitis, asthma and hay fever. Consumption of large amounts of *Parthenium* can be toxic to livestock and can result in the poor quality of milk and tainted meat. It also has a detrimental effect on environment and natural biodiversity.

4.3.1 Impact on Human Health

Parthenium weed is a notorious allergenic plant, and people can become affected either by direct contact or by indirect contact through airborne particles. Persons exposed to *Parthenium* for prolonged period manifest the symptoms of skin inflammation, eczema, asthma, hay fever, black spots, allergic rhinitis, burning and blisters around eyes. *P. hysterophorus* also causes diarrhoea, severe papular erythematous eruptions, breathlessness and choking. Currently, there is no treatment for sensitivity to the plant or its airborne parts, and no desensitising therapies are available. Some allergies can be managed with medications; however, these are not always effective. Dermatitis, hay fever, asthma and bronchitis can be managed with antihistamine medications. Non-contact, allergic respiratory disorders in susceptible people have been reported to have caused some deaths in India. The weed is toxic to livestock and may cause death after 30 days if significant quantities are ingested. Chemicals within the plant are thought to alter the microbial composition of the rumen of dairy cattle, buffalo and sheep and can impart a bitter taste to their milk, and the meat of cattle and sheep can develop an undesirable flavour.

4.3.2 Effect on Livestock Health

Parthenium hysterophorus is known to cause many health hazards which have now reached epidemic proportions. Exposure to *P. hysterophorus* also causes systemic toxicity in livestock. It may cause allergic inflammation of the mouth and udder, rashes on the skin and ulceration of the mouth and digestive tract. Alopecia, loss of skin pigmentation, dermatitis and diarrhoea have also been reported in animals

feeding on *P. hysterophorus*. Degenerative changes in both the liver and kidneys and inhibition of liver dehydrogenases have been reported in buffalo and sheep. When cattle and goats consume the weed, the quality of their milk becomes impaired having an unacceptable odour, and the meat becomes tainted (Tudor et al. 1982).

4.3.3 Damage to Crop Plants

Parthenium weed can invade a wide range of crops. Contamination of rice and wheat seed lots with seed of *Parthenium* can have severe consequences for the export of these crops. Cereal crops such as rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), tef (*Eragrostis tef* Zucc. Trotter) and sorghum (*Sorghum bicolor* L.) grown in different parts of the world can get contaminated and are of major concern. It has been reported to inhibit the growth in a variety of dicot and monocot plants. *Parthenium* enters the soil through the decomposing leaf litter and inhibits germination and radicle growth. Burning *P. hysterophorus* in fields reduced germination of *Phaseolus mungo*, biomass growth, plumule and radicle length (Kumar and Kumar 2010). Fields infested with *Parthenium* showed poor fruiting in leguminous crops and reduction in chlorophyll content. *P. hysterophorus* played role as alternate host for crop pests functioning as an inoculum source. This weed has been reported to serve as a reservoir plant of scarab beetle, a pest of sunflower.

4.3.4 Impact of Parthenium as Secondary Host

Parthenium weed can play as a secondary host to a number of important pests and diseases of various crops and can also cause indirect losses to crop production. These include economically important pests such as the common hairy caterpillar (*Diacrisia oblique* Walk.), *Xanthomonas campestris* pv. Phaseoli and tobacco streak virus. The indirect impacts of *Parthenium* weed on agricultural production are also significant. Other indirect costs include the additional expense incurred by the purchase of herbicides and their application, and labour and machinery hired to help manage the weed.

4.3.5 Effects on Pastures and Native Communities

Contamination with *Parthenium* seed can also affect the marketing of pasture crop seeds. It is a serious invasive weed of pasture systems, reducing pasture productivity 90%. A number of studies have shown that *Parthenium* weed disrupts the

structure of natural ecosystems and displaces numerous native plant species from those ecosystems (Gnanavel 2013). The weed has become a major threat to many protected areas, forest reserves and national parks around the world mainly in Africa, Asia and Australia. It has been reported that *Parthenium* weed can reduce the capacity of pastures by as much as 40% in Australia and up to 90% in India. It squeezes grasslands and pastures, reducing the fodder supply. It has become a major weed of grazing lands in central Queensland and New South Wales in Australia. It is considered to be one of the worst weed species, competing with both native and introduced pasture plants.

4.4 Management

Several approaches including mechanical, chemical, biological as well as cultural have been employed all over the world for control of the spread of this weed. However, most have proven to be either uneconomical or ineffective, and the weed continues to spread and cause losses to crop. Various management approaches are summarized in Table 4.1.

4.4.1 Cultural Management

The plant has been declared as a noxious or prohibited weed in several countries and any incidence of it is supposed to be reported immediately to the government authorities. In many countries, state authorities make the people aware of the economic and environmental threats so that people are careful and act responsibly in case of the weed's occurrence. Farmers of such countries are extra careful with their farm equipments and make sure that they are free from *Parthenium* seeds, etc. In many countries, the weed is quarantined, and product infested with its seeds or adult plants are not allowed to cross into un-infested areas. It is important to have more legislative measures in place to overcome the spread of this noxious weed and even more important to make sure that they are followed. This can only be done when people take it upon themselves to follow the regulations strictly and be careful.

4.4.2 Physical Management

Manual removal of the weed is the most common method of physical management of the weed in many under developed or developing countries; however, it is not feasible due to several restraints such as (1) health hazards associated with the

Table 4.1 Management practices for control of *Parthenium* weed

S. No	Description	References
	Chemical management	
1	Full dose (500 ml/ha) of Glyphosate resulted in up to 100% mortality at 21 days after spray	Shabbir (2013)
2	Full dose of Isoproturon (2 kg/ha) resulted in up to 100% mortality at 21 days after spray	Shabbir (2013)
3	Bromoxynil + 2-Methyl-4-chlorophenoxyacetic acid (recommended dose) resulted in 100% mortality within 7 days	Javaid (2007)
4	Recommended dose of Atrazil 38% SC killed 5 week old plants in 7 days	Javaid (2007)
5	Application of pendimethalin followed by bispyribac-sodium plus bensulfuron-methyl provided the highest growth reduction of 90% *Parthenium* in rice field	Bajwa et al. (2018)
	Biological control	
6	*Cassia sericea* (=*C. uniflora*), *Chromolaena odorata*, *Cassia tora, C. oxydentalis* and *Sida aquata* compete and suppress *Parthenium*	Singh (1983), Abraham and Girija (2005)
7	*Bothriochloa inschupta* (blue grass), *Decanthim aristatum* (floren blue grass), *Cenchrus ciliaris* (bafel grass) and *Clitorea ternatea* (butterfly pea grass) were found to suppress the growth of *Parthenium*	O'Donnell and Adkins (2005)
8	Fungi such as *Entyloma compositarum, Puccinia melampodi* and *Fusarium pallidoroseum* have been found to be host-specific pathogenic fungi for *Parthenium*	Evans (1997), Jaisurya (2005), Kauraw et al. (1997)
9	Bacteria *Ralstonia solanacearum* and *Xanthomanas campestris* attack *Parthenium* but are not host specific	Kishun and Chand (1988)
10	Stem-boring scolytid beetle, *Hypothenamus erudistus* caused wide spread damage to *Parthenium*	Kumar (2009)
11	Detailed host-specificity test of *Z. bicolorata* in Mexico and Australia revealed it as a safe bioagent	McClay (1980), McFadyen (1985)
	Integrated Management	
12	Manual weeding or ploughing as primary tillage followed by mulching with gliricidia leaves as a post-tillage practice suppressed *P. hysterophorus* effectively and enhanced growth and yield of vegetable capsicum	Nishanthan et al. (2018)
13	Pre-emergence application of clomazone + hand-weeding at 40 days of *Partheium* in Soybean field experiment resulted in significantly reduced uptake of nutrition by the weed resulting in increased yield of Soybean	Pandya et al. (2005)
14	A pre-emergence treatment of atrazine (0.75^{-1} ha) with wheat straw mulch (5.0^{-1}) brought about a consistent and significant reduction in the *Parthenium* growth in Sorghum crop	Tadasse et al. (2010)

weed, (2) labour is not cheap in all the countries, (3) fast regeneration of the weed after manual removal due to partial removal or seed dispersion. One of the relatively effective methods could be ploughing of the weed into the soil before the plants start flowering and then establishing the pasture. Burning of the weed was tried in Australia, but it resulted in creation of open niches in the landscape where a large number of seeds were able to germinate in the absence of vegetation. Therefore, this method is not considered feasible.

4.4.3 Chemical Management

Chemical control involves use of weedicides and is feasible only in high-value crops. In marginal lands, forests, wastelands, parks and gardens, where it mostly grows, its use is not economically and environmentally feasible. Even where it can be used, it is important to apply when the weeds are in pre-flowering stages, and also it is important to use selective herbicides which will not affect the main crop and will only target the weed. Thereafter, it is important to monitor the treated area at regular intervals and spot treat wherever and whenever needed. Most commonly used herbicides include clomazone, metribuzin, bentazone, dicamba, atrazine, glyphosate and butachlor. However, chemical herbicides may pose a threat to environment if used for such widely spread growth of the weed, and also after prolonged use, there are possibilities that the weed will develop resistance to them as has been the case with ALS-inhibiting herbicides and glyphosate.

4.4.4 Biological Management

Several metabolites, pathogens, exotic insects and oils containing allelochemicals have been used for biological control of *Parthenium* (Evans 1997; McFadyan 1985). Plants that are able to compete with the growth of *Pathenium* thus limiting growth conditions for the later are also employed in many places. For example, *Cassia sericea* and *Tagetes erecta* have been found to suppress the growth of *P. hysterophorus* growth in field trials. Oils and metabolites containing allelochemicals such as Cassia, Amaranthus, Xanthium, Impereta, Azadirachta have been used for inhibiting seed germination and seedling growth in *Parthenium*. Besides this, natural enemies such as Mexican beetle, seed-feeding and stem-boring weevils, stem-galling and leaf-mining moth, and sap-feeding plant hopper that will affect only the weed and spare the main crop have also been tested for control of the weed. Several metabolites from fungi such as *Fusarium oxysporun* and *Fusarium monilifonne* have also been effective in controlling the weed to some extent.

4.4.5 Integrated Weed Management

None of the methods described above is able to control the spread of the weed effectively. Therefore, a combination of two or more methods is considered most effective way to a long-term weed management. The factors that are considered while designing these combinations include efficiency, economic and environmental sustainability and compatibility. A good example of integrating management methods is intercropping, displacement by competitive plant species like Cassia species, bisset bluegrass, florgen bluegrass, buffelgrass, along with the use of biological control agents like Mexican beetle, seed-feeding and stem-boring weevils, stem-galling and leaf-mining moth, and sap-feeding plant hopper, which has been reported as possible strategy for the management of *Parthenium*.

4.5 Uses of *Parthenium hysterophorus*

In spite of the nuisance caused by apparently unmanageable spread of this weed, the fact that it is able to grow in a broad range of climatic and soil conditions and produces a huge amount of biomass, needs to be exploited to our benefit. Several studies have indicated that the weed can be useful for making of various value-added products (Saini et al. 2014). Besides this, the allelochemicals present in *Parthenium* can be separated and put to use for fighting plant and animal diseases. There are also reports of use of *Parthenium* in treatment of various diseases (Table 4.2). The following section discusses possible uses and various studies that explore the uses of this weed.

4.5.1 Allelochemicals

Chemical analysis of *P. hysterophorus* has indicated that all its parts including trichomes and pollen contain toxins called sesquiterpene lactones (SQL). Maishi et al. (1998) reported that *P. hysterophorus* contains a bitter glycoside parthenin, a major sesquiterpene lactone. Other phytotoxic compounds or allelochemicals are hysterin, ambrosin, flavonoids such as quercelagetin 3,7-dimethylether, 6-hydroxyl kaempferol 3-0 arabinoglucoside and fumaric acid. Plants that consist of allelochemicals are allelopathic in nature. That means the chemicals produced by them influence the germination, growth survival and reproduction of other plants in the immediate environment. The invasive nature of *Parthenium* is largely due to these allelochemicals. However, when isolated, these allelochemicals can be used as herbicides and many other products.

Table 4.2 Applications of *Parthenium* weed

S. No	Description	References
	Bioremediation	
1	Adsorption of nickel and cadmium ions from dilute aqueous solution	Lata et al. (2008), Ajmal et al. (2006)
2	Absorption of p-Cresolin aqueous medium	Singh et al. (2008)
3	Removal of methylene blue dye from aqueous solution	Lata et al. (2007)
	Green Manuring and Biochar production	
4	High content of N, P and K	Javaid and Shah (2008)
5	Higher nitrogen content than poultry manure, vermicompost or Farm Yard Manure	Channappagoudar et al. (2007)
6	Feedstock for vermicomposting	Yadav and Garg (2011)
7	Use of *P. hyterophorus* compost lowers the weed population in rice field due to allelopathic effect of compounds present in it	Belz et al. (2007)
8	*Parthenium* biochar can improve soil structure, water and nutrient holding capacity and C/N ratio and improves agronomic performance of rice-wheat system	Qurat-ul et al. (2016)
9	Addition of *Parthenium* biochar to soil increased the soil dehydrogenase and catalase activity while decreased the hydrolytic enzyme activity. The *Zea mays* seeds sown in such soil showed increased seedling vigour	Kumar et al. (2013)
	Pesticidal effect	
10	Sesquiterpene lactones from *Parthenium* inhibit the growth of *Heliothis zea* insects	Isman and Rodriguez (1983)
11	Roots and stems of *P. hysterophorous* cause the mortality of mosquito larvae	Kumar et al. (2011)
12	Antifungal and antibacterial properties	Joshi et al. (2016)
13	Ovicidal, anti-fleedant and nematocidal activity	Datta and Saxena (2001)
	Medicinal properties	
14	Anticancer activity of *Parthenium* phenolic extract against A-498 (IC50 0.5237 ìg/ml) and MDA-MB231 (IC50 and 0.2685 ìg/ml) cancerous cell lines	Panwar et al. (2015)
15	Treatment of insomnia by pouring its drops in eyes	Maishi et al. (1998)
16	Treat inflammation, eczema, skin rashes, herpes, rheumatic pain, cold heart trouble and as a remedy for female ailments	Surib-Fakim et al. (1996)
	Nanomaterial	
18	Antifungal zinc oxide nanoparticles from *P. hysterophorus* leaves	Rajiv et al. (2013)
19	Silver nanoparticles from aqueous root extract of *P. hysterophorus* have application in mosquito control	Mondal et al. (2014)

(continued)

Table 4.2 (continued)

S. No	Description	References
20	Silver nanoparticles prepared from digested *Parthenium* slurry show antibacterial activity	Adur et al. (2018)
	Biofuel	
21	*Parthenium hysterophorus* is at par with agro- and forest residues as biofuels feedstock	Singh et al. (2014)
22	0.24–0.27 g of ethanol/gram of *P. hysterophorus* was achieved	Tavva et al. (2016)
23	Production of cellulases and xylanases under solid state fermentation of *P. hysterophorus*	Bharti et al. (2018)
24	A significant increase in methane content, i.e. up to 60–70%, was achieved when 10% of weed is mixed with the cow dung	Gunaseelan (1987)

4.5.2 *In Agriculture*

Parthenium produces huge amount of biomass that can be used for green manuring (Javaid and Shafique 2009; Saravanane et al. 2008, 2012). The green leaf manure when added to paddy fields was able to increase the height of the plant, yield of grain and straw and also ensured inhibition of growth of weeds in the submerged plantation. Its addition also increased the number of filled grains in ratoon rice crop. Similarly, Maize crop shows higher assimilation rate of nitrogen and phosphorus and enhancement in growth on addition of *P. hysterophorus* green manure. On an average, the need for chemical fertilizers is reduced by about 25% on addition of *Parthenium* green manure. In a study involving a combination of *P. hysterophorus* green manure (Suryawanshi 2011) with effective microorganisms (EM), a biofertilizer resulted in higher root biomass in 3% green manure-amended treatment (Javaid and Shah 2008). *Parthenium* can also be added to cow dung for vermicomposting (Yadav and Garg 2011). Doing so led to an increase in electrical conductivity, nitrogen, potassium, calcium and phosphorus. Compost from this weed on application in soil can enhance its moisture level more than nitrogen, phosphorus and potassium (NPK) alone (Kishor et al. 2010). Thermal combustion of *Parthenium* converts it into biochar which can be used for improving soil fertility and for many other purposes. It was found that combustion at 300–350 °C temperate with 30–45 min residential time led to higher stable organic matter yield index (SOMYI) and addition of biochar thus prepared improved the soil microbial activity (Kumar et al. 2013). *Parthenium* biochar can also improve soil structure, water, nutrient holding capacity and C/N ratio. The allelochemicals can be used for the enhancement of crop productivity by exploiting them as herbicides, insecticides, nematicides, fungicides and growth regulators (Raju et al. 2013). The allelochemicals also provide defence against herbivorous predators. The isolated sesquiterpenes have been found to have activity against insects, larvae and various pathogenic microbes.

4.5.3 In Health and Medicine

The extracts and decoctions prepared from various parts of *Parthenium* have been used in traditional and folk medicines since a long time. Its root extract is useful in treating dysentery (Singh et al. 1996), while other parts' extracts are known to be applied for skin ailments, as flea repellent for dogs and other animals (Morton 1981). In the countries like West Indies and Central America, it is used as a folk remedy for various diseases such as inflammation, rheumatic pain, skin rashes and as an analgesic in muscular pain, neuralgia, etc., (Navie et al. 1996; Maishi et al. 1998), and its decoction is even taken internally as a remedy for a wide variety of ailments. It is also reported to be a promising treatment for hepatic amoebiasis (Sharma and Bhutani 1988). The major sesquiterpene present in *Parthenium*, parthenin exhibits anticancer property (Venkataiah et al. 2003), and a methanol extract of its flowers was found to have antitumour potential (Ramos et al. 2002). An aqueous extract of flowers was shown to have hypoglycaemic activity against alloxan induced diabetes in rats (Patel et al. 2008). The high amounts of potash, oxalic acid and high quality protein in *Parthenium* make it an excellent additive in feed for livestock (Mane et al. 1986).

4.5.4 In Industry

The lignocellulosic biomass obtained from *P. hysterophorus* consists of approximately 28% cellulose, 21% hemicelluloses and 13–17% lignin. Cellulose is used for making paper, and therefore, *Parthenium* can be readily used for making paper of various strengths and qualities (Naithani et al. 2008). It has also been successfully used as a substrate for production of glycoside hydrolases such as xylanases and cellulases in solid state fermentation and displayed maximum endo-glucanase, xylanase and FPase activities. This implies that the biomass of this weed can serve as a low-cost substrate for production of these industrially important enzymes and the products such as cellulose and hemicelluloses produced in the process can be further used for production of other value-added commodities. These by-products can be further derivatized to make compounds such as carboxymethyl cellulose (CMC), cyanoethyl cellulose (CEC), hydroxymethyl cellulose (HMC), ethyl cellulose (EC) that have application in textile, paint, pharmaceutical, cosmetic, food, adhesives and packaging industries. Not only this, the weed has found use in bioremediation of waste generated by industries. The effluents of industries like silver refineries, electroplating, zinc base casting and storage battery industries consist of nickel (II) which at higher concentrations, can cause cancer of lungs, nose and bone. Lata et al. (2008) found that sulphuric acid treated *Parthenium* can be used as an efficient and low-cost adsorbent for nickel (II) in dilute aqueous solutions. Similarly, dried and powdered *Parthenium* proved to be an efficient adsorbent for cadmium (II) (Ajmal et al. 2006) which is extremely toxic even in low concentrations and is

widely used in electroplating, plastic manufacturing, metallurgical processes and industries of pigments and Cd/Ni batteries. Activated carbon prepared from *P. hysterophorous* can also be used for remediation of effluent laden with p-cresol which is a phenol derivative found in effluents of petrochemical, oil and metal refineries, chemical and glass fibre manufacturing, ceramic and steel plants, phenolic resin manufacturing industries, etc. It is known to cause several health hazards including stomach tumours, corrosion of eyes, skin and respiratory tract, etc.

4.5.5 In Biofuels

Fuel is one of the basic needs of industrial and societal development. Biofuel research has gained considerable interest in the recent past due the fact that these are renewable fuels and are considered environmentally friendlier compared to their conventional counterparts. Several different sources have been explored for production of biofuels including edible starch, edible oils, lignocellulosic biomass, microbial production, algal fuel, etc. In spite of the many environmental benefits offered by biofuels derived from edible sources, they lose out in the food versus fuel debate. Cellulosic ethanol, which is derived from ligno cellulosic biomass, has emerged as the most viable option in terms of cost and availability of raw material (Ratnaparkhe et al. 2016). *Parthenium* offers an excellent source for biofuel production due to its high cellulosic content and other benefits such as fast growth, ability to grow on marginal lands thereby eliminating the need for utilization of arable land for growing biofuel crops, and the ability to grow in wide range of climatic and soil condition, thereby ensuring uninterrupted supply of raw material for biofuel industry. However, in order to utilize the weed for production of bioethanol, it is important to design an optimum pre-treatment technology in order to free as much total fermentable sugar as possible. Shubhaneel Neogi (2013) reported 61.7 mg/g of xylose yield on hydrolysis of dry Parthenium with 5% H_2SO_4 at a temperature of 121 °C. Autoclaving *Parthenium* biomass at 121 °C and 15 psi pressure for 30 min in acidic (1% v/v, H_2SO_4) environment yielded 397.7 mg/g of total fermentable sugars (Singh et al. 2014), while a pre-treatment with each of the three acids (hydrochloric, sulphuric and phosphoric) and two alkalis (sodium hydroxide and potassium hydroxide) under two temperature regimen (cold hydrolysis at room temperature and hot hydrolysis at 121 °C) followed by detoxification of inhibitors yielded 615 mg/g total reducible sugars to eventually produce 0.27 g/g of ethanol (Tavva et al. 2016). There are also reports of using *Parthenium* for biogas production. A substrate mix with a ratio of 75% *Parthenium* with 25% poultry manure proved to be optimal mix for biogas production (Egigu 2014).

4.5.6 In Generation of Nanoparticles

The use of environmentally benign materials like plant leaf extract, bacteria and fungi for the synthesis of silver nanoparticles offers numerous benefits of

eco-friendliness and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols (Kumar et al. 2012; Mangrola et al. 2012). Chemical synthesis methods lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in medical applications. Bio-inspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and environment friendly, and in this method, there is no need to use high pressure, energy, temperature and toxic chemicals. Parashar et al. (2009) reported synthesis of silver nanoparticles, reducing silver ions present in the aqueous solution of silver nitrate complex from the extract of *P. hysterophorus* L. leaves. Their research indicates that it will help work in the development of value-added products from *Parthenium* for biomedical and nanotechnology-based industries.

4.6 Conclusion and Future Prospects

Limited work related to application of *Parthenium* for useful purposes exists. Besides this, most of the work done in this regard is at laboratory scale. In order to realize the fact that *Parthenium* can be used for making value-added commodities, scalable technologies will need to be designed and practised. Harvesting the *Parthenium* has several desirable properties that can be exploited in or favour, and this will actually be the best management practice for this plant which otherwise is regarded as noxious weed. *Parthenium* has many utilities, each of which can be used separately to control this weed. Such methods can also be designed in future, which integrate two or more applications, aiming at maximum utilization of weed. Thus, new and improved methods of managing *P. hysterophorus* weed, encouraging well-being of human society, are anticipated in near future.

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Part III
Weed Utilization

Chapter 5
Seaweed Biomass Utilization Pathways in Microbes and Their Applications in the Production of Biofuels

Sujit Sadashiv Jagtap and Ashwini Ashok Bedekar

5.1 Introduction

Marine algae transform nearly 50 Gt of carbon dioxide each year from the atmosphere and convert it into biomass (Falkowski et al. 1998). Carbohydrates are the main components of the algal biomass, which function as storage of carbon and energy (Hehemann et al. 2010a, 2014). Carbohydrates also present 30–80% of the overall carbon content in algal biomass (Alderkamp et al. 2007). Microorganisms have the ability to convert a wide variety of carbohydrates produced by macroalgae in the ocean. Interestingly, most of the enzymes involved in the marine carbohydrates cycle are still unknown.

Macroalgae show numerous features of a potential feedstock that may help the increasing global requirement for energy. The farming of macroalgae does not require fertile land, freshwater, use of fertilizer, avoiding adversarial impacts on food supplies (Enquist-Newman et al. 2013). Additionally, algal polysaccharides are simpler to digest than terrestrial plant biomass (Horn et al. 2000a; Singh et al. 2017). Also, they are deficient or comprise minor quantities of lignin (Martone et al. 2009), the natural part that reduces cellulose degradation by cellulase. Macroalgae are classified into three main groups brown, red, and green. There are characteristic differences between each type in carbohydrate composition.

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In brown macroalgae, alginate, glucose, and mannitol are present in ample amount. Alginate is consumed by sea microbes (Mitulla et al. 2016). Alginate is one of the plentiful sea polysaccharide and is located in the cell walls of brown macroalgae (Smith 1981). Alginate is a polymer of two uronic acids, β-D-mannuronate (M), and α-L-guluronate (G). They are arranged in variable orders of poly β-D-mannuronate (polyM), poly α-L-guluronate (polyG), and the heteropolymer (polyMG) (Wong et al. 2000). Native alginate metabolism pathways have been reported in several microorganisms (Cao et al. 2007).

Microorganisms need several enzymes to decompose even the simplest polysaccharides into simple sugars. These enzymes involve hydrolytic glycoside hydrolases and lytic polysaccharide lyases (PL). Enzymes essential to deconstruct a variety of algal polysaccharides have been reported earlier (Hehemann et al. 2012b, c; Thomas et al. 2012). Alginate degrading microorganisms contain alginate lyases (Alys) which degrade the alginate via a β-elimination reaction (Wargacki et al. 2012). Alginate lyases from different microorganisms have been well explored (Thomas et al. 2012; Wong et al. 2000). Alginate lyases initiate the reaction by cleaving the polymer into longer oligosaccharides in an endo-mode of action. Oligoalginate lyases act in exo-mode from the ends of oligomers and polymers to convert them into monomer (Wong et al. 2000). Oligoalginate lyases eliminate unsaturated and saturated monomers from the nonreducing end of alginate polymers (Gimmestad et al. 2009; Suzuki et al. 2006).

Seaweed polysaccharides have been considered as cheap biomass to produce biodiesel, ethanol, and hydrogen (Beer et al. 2009; Chisti 2008; Giri and Pant 2019). Alginate has been projected as a sustainable source to produce ethanol (Wargacki et al. 2012; Zimmerman et al. 2013). The alginate degradation pathway has been transferred from the marine bacterium *Vibrio splendidus* 12B01 into an *Escherichia coli* and *Saccharomyces cerevisiae*. These hosts are further engineered to produce bioethanol from alginate (Wargacki et al. 2012).

This book chapter is focused on macroalgae-based biorefinery. It provides a background on macroalgae taxonomic classification, habitat environment, enzymes, and metabolic pathways involved in macroalgae polysaccharide catabolism. In addition, it is also focused on providing information on native and engineered microbial platforms for biofuel production from brown macroalgae.

5.2 Classification and Habitat of Macroalgae

Macroalgae are multicellular photosynthetic organisms made up of a leaf-like structure (Jung et al. 2013; Lobban et al. 1985). On the basis of pigment present in thallus, macroalgae are classified as green, brown, and red algae (Sze 1993) (Fig. 5.1). There are about 3050 and 1500 species of freshwater and seawater green macroalgae, respectively (Guiry 2014). Their composition is similar to land plants (Yu et al. 2002). More than 4000 species of red macroalgae and 2000 species of

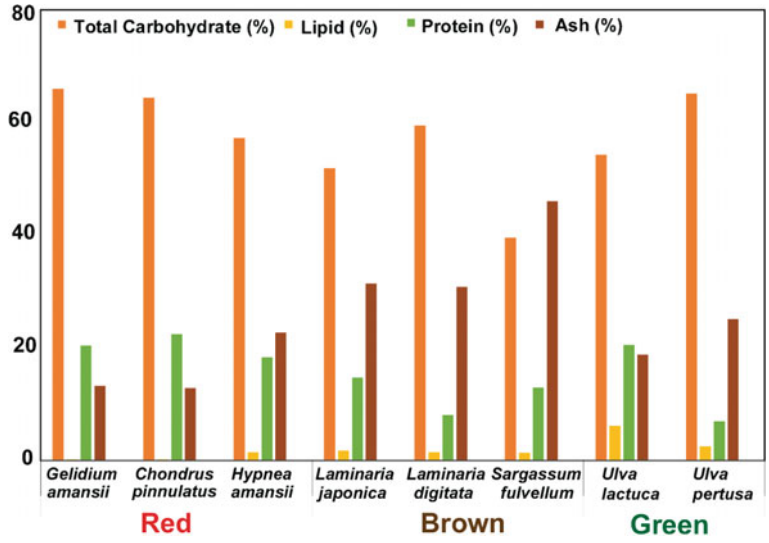

Fig. 5.1 Total composition of green, brown, and red algae

brown macroalgae are exist in seawater (Hoek et al. 1995). The brown color is derived from chlorophyll, β-carotene, and xanthophyll pigments (Sze 1993).

The growth, pigments, and element structure of macroalgae are disturbed by habitat surroundings including nutrient, salinity, temperature, water motion, and light (Lobban et al. 1985). Light availability is a key contributing condition in the distribution of macroalgae (Choi et al. 2019; Patel et al. 2019). The specific pigment in macroalgae absorbs light with a particular wavelength (Guiry 2014; Pant et al. 2018).

5.3 Carbon Storage Potential of Macroalgae

Organic carbon is stored in photoautotrophic macroalgae by consuming CO_2 or HCO_3^- (Gao and McKinley 1994). The intake of HCO_3^- is preferred over CO_2 by macroalgae. The mass transfer rate of CO_2 in seawater is very slow (Giri and Pant 2019, 2020). A small number of macroalgae can directly use CO_2 as a substrate. Interconversion of CO_2 and HCO_3^- is catalyzed by RuBP carboxylase and carbonic anhydrase (Lobban et al. 1985).

Macroalgae can store carbon resources required for the production of biochemicals (Bhatia et al. 2019). The green and red macroalgae have a higher photosynthetic rate as compared to brown macroalgae. The 1 billion tons of carbon could sequestrate by macroalgae cultivation along with coastlines. Brown and red macroalgae are the most favorable species for biorefinery. *Laminaria japonica* and

Undaria pinnatifida are widely produced brown macroalgae. In red seaweed, Eucheuma spp.*, Kappaphycus alvarezii, and Gracilaria verrucosa constitute more than 40%.*

5.4 Composition of Macroalgae

The seaweed composition is significantly different from lignocellulosic biomass. Lignin is absent in macroalgae. Uranic acids, sugars, and sugar alcohol mannitol are more prevalent in seaweed (Table 5.1). Macroalgae are made up of 70–90% water per wet weight. They contain 10–50% alkali metals, 7–15% protein, and 1–5% lipids per dry weight (Ross et al. 2008). The 25–50, 30–60, and 30–50% carbohydrates are existing in green, red, and brown macroalgae, respectively (Becker 1994; Ross et al. 2008).

5.4.1 Green Macroalgae

The 1–4% starch and 0–6% lipids are the main polysaccharides in green macroalgae (Burton et al. 2009). *Ulva* and *Enteromorpha* sp. composed of ulvan and cellulose in their cell wall. Ulvan is comprised of D-glucuronic acid, xylose, rhamnose, and sulfate (Lahaye and Robic 2007).

Table 5.1 Carbohydrate and sugar composition of different algae

Class	Carbohydrate composition	Sugar composition
Red	Cellulose	Glucose
	Agarose	Galactose
	Carrageenan	
	Starch	
Brown	Laminarin	Glucose
	Mannitol	Fucose
	Alginate	Mannitol
	Fucoidan	Mannuronic acid
		Guluronic acid
Green	Cellulose	Glucose
	Ulvan	Mannose
	Starch	Uronic acid
	Mannan	Rhamnose
	Xyloglucan	Xylose
		Glucuronic acid

5.4.2 Red Macroalgae

The unique characteristic features of red macroalgae are floridean starch and floridoside. These carbohydrates are absent in green and brown macroalgae. Floridean starch is glucose homopolymer and accounts up to 70% of cell volume (Yu et al. 2002). Agar and carrageenan are the galactans and major polysaccharide constituents of red seaweed (McHugh 2003). Carrageenan contains a repeating unit of galactose and anhydrogalactose, with or without sulfate. Agar consists of interchanging β-D-galactose and α-L-galactose with limited sulfations (Jung et al. 2013; Lobban et al. 1985).

5.4.3 Brown Macroalgae

Alginate, laminarin, mannitol, and fucoidan are ample sugars in brown macroalgae (Table 5.2). Alginate is a major carbohydrate in brown macroalgae and is composed of β-1,4-D-mannuronate and α-1,4-L-guluronate residues (Davis et al. 2003; Usov et al. 2001). Alginate degradation is important for the efficient conversion of brown macroalgae. Laminarin is the main storage polysaccharide composed of a linear β-1,3-D-glucose chain with scattered branches of β-1,6-D-glucose. Mannitol is an alcohol polymer of mannose. Fucoidan is composed of fucose and sulfate. In addition, it also comprises galactose, xylose, glucuronic, and uronic acid (Li et al. 2008).

5.5 Pretreatment Technologies

The various pretreatment technologies have been used to release sugars from macroalgae comprising, physical, chemical, biological, or a combination of methods (Milledge et al. 2019). The physical structure of seaweeds can be disrupted by mechanical pretreatment. It enhances the hydrolysis of macroalgae to sugars for fermentation. Different mechanical treatments are used for macroalgae pretreatment

Table 5.2 Composition of brown algae

Brown algae	Alginate (%)	Laminarin (%)	Fucoidan (%)	Mannitol (%)
Laminaria digitata	16–45	0–18	2–4	4–22
Saccharina latissima	21–46	0–26	–	6–22
Laminaria hyperborean	22–35	0–24	2–4	6–18
Ascophyllum nodosum	15–30	0–10	5–10	5–10
Fucus vesiculoses	14–17	2–5	–	8–16

including a Hollander beating, size reduction by chopping, washing in freshwater to remove impurities, and sonication. Thermal pretreatment is used to release sugars from macroalgae. Bioethanol yields are increased when autoclave treatment used for brown, red, and green macroalgae. H_2SO_4 and NaOH are most commonly proposed for acid and alkali treatments. It causes swelling of macroalgae fibers and increases pore size to release sugars (Jagtap et al. 2013, 2014a). Ionic liquids and sodium chlorite have also been used as pretreatment methods. In biological pretreatment, enzymes or microbes are used for the conversion of macroalgae to sugars. Macroalgae degrading enzymes and commercial enzymes, such as Celluclast 1.5L, are most commonly used for the conversion of macroalgae. The widely used pretreatment method is hydrothermal treatment with acid or alkali (Kim et al. 2013). It releases inhibitors furfural, 5-hydroxymethylfurfural (5-HMF), and levulinic acid along with sugars (Martín et al. 2002). An eco-friendly gamma radiation method has been also reported as an effective pretreatment method (Yoon et al. 2012).

5.6 Enzymes for Seaweed Conversion and Mechanism of Action

5.6.1 Laminarinase and Fucoidanases

Laminarinase and fucoidanases belong to glycoside hydrolyase (Badur et al. 2020; Becker et al. 2017, 2020). They break β-1,3-glycosidic linkages and β-1,6-glycosidic linkages of laminarin in one step or two steps reactions. Laminarinase is functional on the β-1,3-linked substrates including curdlan, laminarin, and lichenan. The glycoside bonds between sulfated fucose residues are cleaved by fucoidanases (Kusaykin et al. 2015).

5.6.2 Agarase and Carrageenanase

Agarase catalyzes the hydrolysis of polysaccharide agar. Agarases are categorized as α-agarase or β-agarase depending on the cleavage pattern. The cleavage of α-1,3 linkages by α-agarase produces agarooligosaccharides associated with agarobiose. β-agarases cleaves β-1,4 linkages to generate neoagarooligosaccharides linked to neoagarobiose (Fu and Kim 2010; Hehemann et al. 2012a, b; Pluvinage et al. 2013).

Carrageenan is a sulfated carbohydrate composed of galactose and 3, 6-anhydrogalactose. It is categorized by the number and the location of sulfated esters including κ-, ι- and λ-carrageenan. Carrageenases are endohydrolases that cleave the internal β-(1-4) linkages of carrageenans to generate the

oligocarrageenans (Chauhan and Saxena 2016). Carrageenases are evolved and adapted to degrade the different forms of carrageen (Hettle et al. 2019; Pluvinage et al. 2013).

5.6.3 Alginate Lyases

Alginate lyase is categorized based on the conversion of polyM, polyG, or polyMG regions of alginate (Fig. 5.2). They belong to the 23 protein families of polysaccharide lyases (Murata et al. 2008). Alginate lyases belong to the PL5 to PL7, PL14 to PL15, and PL17 to PL18 families (Yamasaki et al. 2005). PL5 and PL7 alginate lyases act on polymers in endolytic fashion and exolytic fashion to generate smaller oligomers and monomers (Thomas et al. 2013).

Few bacteria can produce alginate, and alginate lyase for alginate degradation including *Pseudomonas aeruginosa* and *Azotobacter vinelandii* (Ertesvåg 2015; Gimmestad et al. 2009). They can be secreted outside, bound to the membrane, or intracellular. Several bacteria use a number of alginate lyases to degrade alginate polymer (Neumann et al. 2015). *Saccharophagus degradans* 2–40 has 13 predicted genes encoding alginate lyase (Hutcheson et al. 2011). *Alteromonadales Zobellia galactanivorans* also processes multiple alginate lyases (Zhu et al. 2017).

V. splendidus 12B01 represents the most abundant *vibrio* of temperate waters. Several alginate lyases within a single *vibrio* have been assessed which provide a keen understanding of how *vibrio* can proficiently use alginate as its main carbon source and potentially inform the strategy for creating new organisms efficient of making biofuels from alginate.

Alginate lyases belong to *vibrio* showed an optimal pH 7.5–8.5, an optimal temperature 20–25 °C, and an optimal NaCl 400–1000 mM (Badur et al. 2015). AlyB, AlyD, and AlyE were reported to have signal peptides and different substrate specificity.

5.6.4 Oligoalginate Lyases

The oligoalginate lyases from *V. splendidus* 12B01 alginate degradation pathway belong to PL15 (OalA) and PL17 (OalB, and OalC) families (Wargacki et al. 2012). Their functional characterization has been reported earlier (Jagtap et al. 2014b) (Fig. 5.2). These enzymes have complementary functions regarding substrate scope and physiological adaptations. They also showed different kinetic rates of alginate degradation.

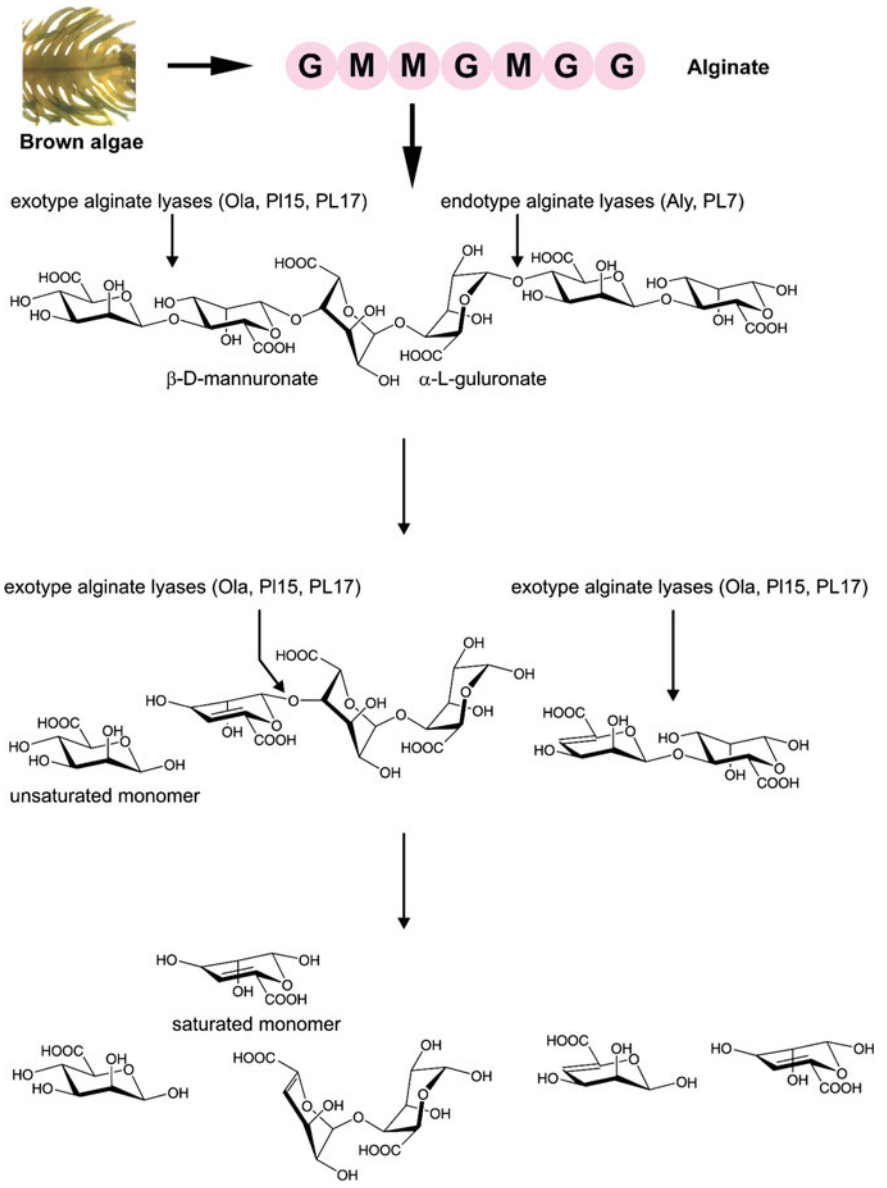

Fig. 5.2 Alginate lyase catalyzed degradation of alginate. Exolytic oligoalginate lyases (PL15 and PL 18) degrades alginate via a β-elimination reaction to monosaccharides in an exolytic manner

5.6.5 Complementary Oligoalginate Lyases

The harmonizing substrate choice was observed for oligoalginate lyases from *V. splendidus*. In addition, complementary role is also observed in optimum temperature and optimum pH adaptations. In the coastal marine environment, bacteria are exposed to high temperature and pH fluctuations. Thus, the temperature range covered with three Oal's increase enzymatic activity at different temperatures. Altogether, the broad substrate scope and the physiological adaptations explained the presence of multiple oligoalginate lyases in 12B01.

5.6.6 Mechanism of Action

Carbohydrate active enzymes use diverse mechanisms to degrade agars, alginate, and ulvan polysaccharides from red, brown, and green macroalgae, respectively (Hehemann et al. 2014). β-agarase is fitted to GH16, GH50, GH85, GH118 families. It uses two catalytic glutamate residues for hydrolysis of agar. α-agarase is a part of families GH96 and GH117. It uses histidine as a catalytic acid and aspartate as a base for catalysis. Brown macroalgae are digested by polysaccharide lyase which belongs to families PL7, PL14, PL15, and PL17. Alginate lyases can either have endo- or exo-acting specificity on alginate polymer (Wong et al. 2000). The mechanism of alginate degradation has been previously suggested (Linker et al. 1960), which includes a positive residue that steadies the negative charge on the carboxyl group, residue operating as a general base abstracts the proton from C-5 of the sugar ring, and acid residue protonates the glycosidic bond oxygen. Ulvan lyases belong to family GH105. The catalytic hydrolysis happens through the addition of water to unsaturated bond for a cleavage of glycosidic bond.

5.7 Alginate Metabolism in *V. splendidus*

In *V. splendidus* 12B01, alginate lyase gradually converts alginate polymer into oligomers with chain length of 2–6 m. Oligoalginate lyases rapidly convert these smaller oligomers into the monomers. These monomers naturally rearrange into 4-deoxy-L-erythro-5-hexoseulose uronic acid (DEH). Consequently, DEH reductase (DEHR) reduces DEH into 2-keto-3-deoxygluconate (KDG), a shared metabolite that enters into the Entner-Doudoroff (ED) pathway. KDG finally resulted in yielding pyruvate and glyceraldehyde-3-phosphate through the activities of the KDG kinase (KDGK) and KDG-6-phosphate aldolase (Eda) and finally produced precursor for the biofuel synthesis (Wargacki et al. 2012).

The capability of alginate degradation is investigated in diverse closely related marine *Vibrionaceae* bacteria (Hehemann et al. 2016). These bacteria can degrade

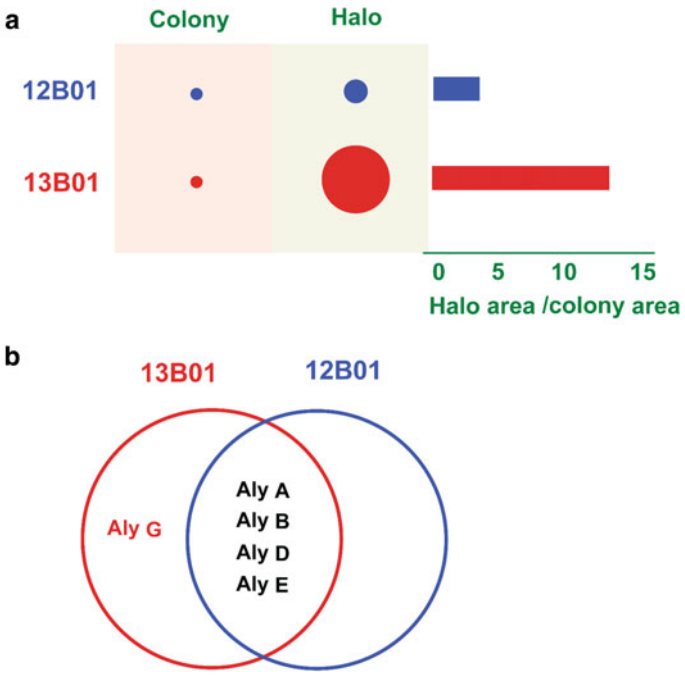

Fig. 5.3 Comparison of secreted alginate lyase activity among closely related *Vibrio splendidus* strains. **a** Phylogenetic comparison of closely related *V. splendidus* strains. The dark halos indicative of alginate digested by secreted alginate lyase. **b** Venn diagram analysis of the alginate lyases from *V. splendidus* 13B01 and *V. splendidus* 12B01

extracellular alginate to variable degrees of oligomers. *V. splendidus* 13B01 has significantly greater secreted alginate lyase activity than *V. splendidus* 12B01 (Badur et al. 2015). Genomic comparison discovered dissimilarities between alginate lyases in *V. splendidus* strains (Fig. 5.3). Both *V. splendidus* strains possessed four alginate lyases (PL7A, PL7B, PL7D, and PL7E) involved in alginate metabolism. Interestingly, PL7G alginate lyase is present in *V. splendidus* 13B01 only, but not in 12B01. The key role of PL7G alginate lyase for fast extracellular alginate degradation is investigated using a combination of different approaches (Ahmet et al. 2017).

5.8 Alginate Degradation Pathway in *Sphingomonas* sp. A1

Sphingomonas genus bacteria are gram negative, yellow colored, rod shaped, and aerobic in nature. *Sphingomonas* sp. A1 has superchannel or pit on the cell surface to import alginate without degradation. Mouth like pit is formed by *Sphingomonas* sp. A1 cells when grown on alginate plus medium (Hashimoto et al.

2010; White et al. 1996). The alginate operon is involved in alginate incorporation by strain A1 cells (Fig. 5.5). The five genes operon is assembled in the genome encodes for the ATP-binding proteins (AlgS/AlgS), transmembrane domains (AlgM1 and AlgM2), and two alginate-binding proteins (AlgQ1 and AlgQ2). AlgQ1 and AlgQ2 are inducibly expressed in the periplasm of strain A1. ABC transporter is constituted of AlgS as an ATPase and AlgM1 and AlgM2 as a permease. AlgQ1-AlgQ2 opened widely after alginate binding to release alginate. The AlgM1 and AlgM2 inducibly activated the periplasmic entrance. Subsequently, AlgS is found to be incorporating the alginate to the cytoplasm (Hashimoto et al. 2010).

Sphingomonas sp. A1 produced the three cytoplasmic endolytic alginate lyases including A1-I, A1-II, and A1-III. The cleavage of N terminal peptide of the precursor protein (Po) leads to synthesis of A1-I. It displayed the affinity for both polyM and polyG. A1-I includes N terminal A1-III and C terminal A1-II. They are processed to generate A1-III and A1-II for polyG and polyM. Alginate is degraded by A1-I, A1-II, and A1-III to unsaturated oligomers. These saturated and unsaturated oligosaccharides are converted to monosaccharides by oligoalginate lyases A1-IV (Hashimoto et al. 2000). The unsaturated uronic acid is spontaneously converted to DEH, which further reduced to 2-keto-3-deoxy-D-gluconate and metabolized into pyruvate (Fig. 5.4). Bioethanol has been produced by the engineering of the strain A1 metabolism (Takeda et al. 2011).

5.9 Brown Macroalgae Degradation Pathway in Engineered *E. coli*

The rapid engineering of native organisms has bottlenecks for the production and optimization of desired product and minimization of byproducts. These efforts were also prevented by a scarcity of tools for genetic engineering and the absence of sturdiness under modern fermentation conditions (Alper and Stephanopoulos 2009). *E. coli* is engineered for alginate catabolism (Fig. 5.5). The secreatable Aly system is engineered to allow effective and fast conversion of alginate (Wargacki et al. 2012). Alginate polymer converted into oligomers by several alginate lyases. Oligomers are transferred through the outer-membrane porins (KDGMN) into the periplasmic place. Subsequently, periplasmic alginate lyases (AlyABCD) converted these oligomers with a degree of polymerization (DP) larger than 3 into dimer, trimer, and tetramers. Oligomers are transported into the cytoplasm via oligoalginate transporters (ToaABC). Oligomers are further converted into unsaturated monomers by oligoalginate lyase (OalABC). These monomers naturally reorganized into DEH. Subsequently, DEHR was reported to reduce DEH into KDG, which entered the ED pathway (Fig. 5.5). KDG finally produced pyruvate and glyceraldehyde-3-phosphate through the actions of the KDGK and KDG-6-phosphate aldolase (Eda) (Wargacki et al. 2012).

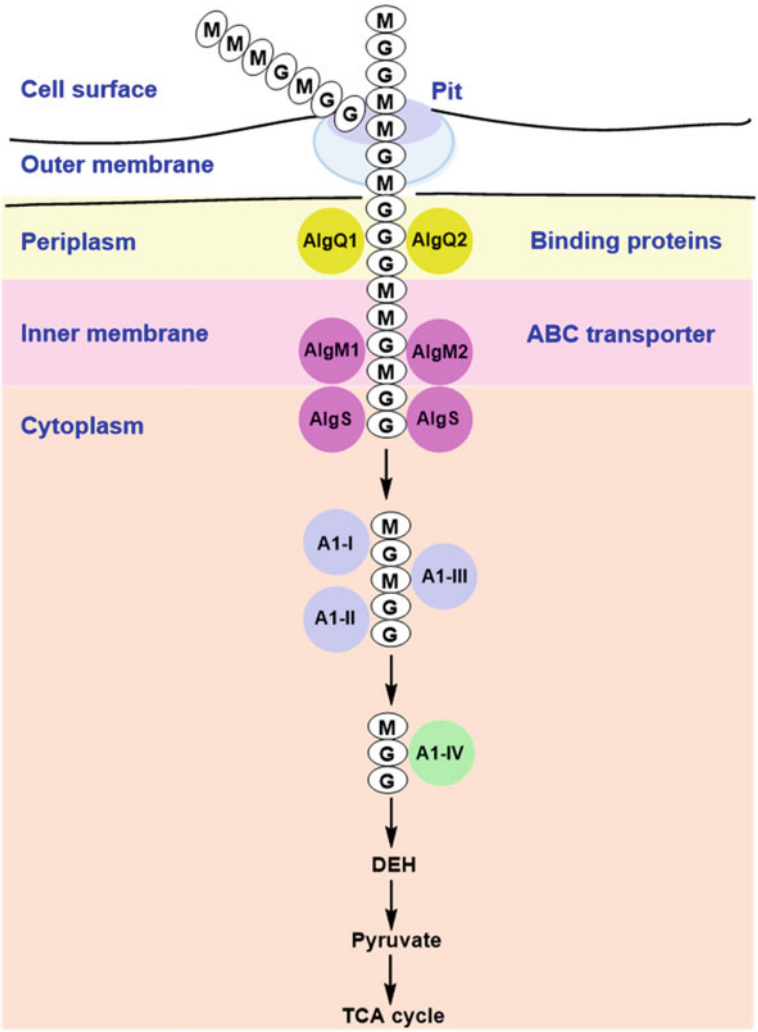

Fig. 5.4 Alginate uptake and degradation system in *Sphingomonas* sp. strain A1. G, L-guluronate; M, D-mannuronate; alginate lyases (A1-I, A1-II, and A1-III); AlgS, AlgM1, and AlgM2, ABC transporter genes for alginate import; AlgQ1 and AlgQ2, alginate-binding proteins; A1-IV, oligoalginate lyase

The alginate metabolic pathway was constructed using a fosmid library of arbitrary DNA pieces using the genomic DNA of *V. splendidus*. The 40-kbp fragment is composed of everything necessary for alginate transport and metabolism. The expression of supplementary genes encoding KDGMN, ToaC, and Alys

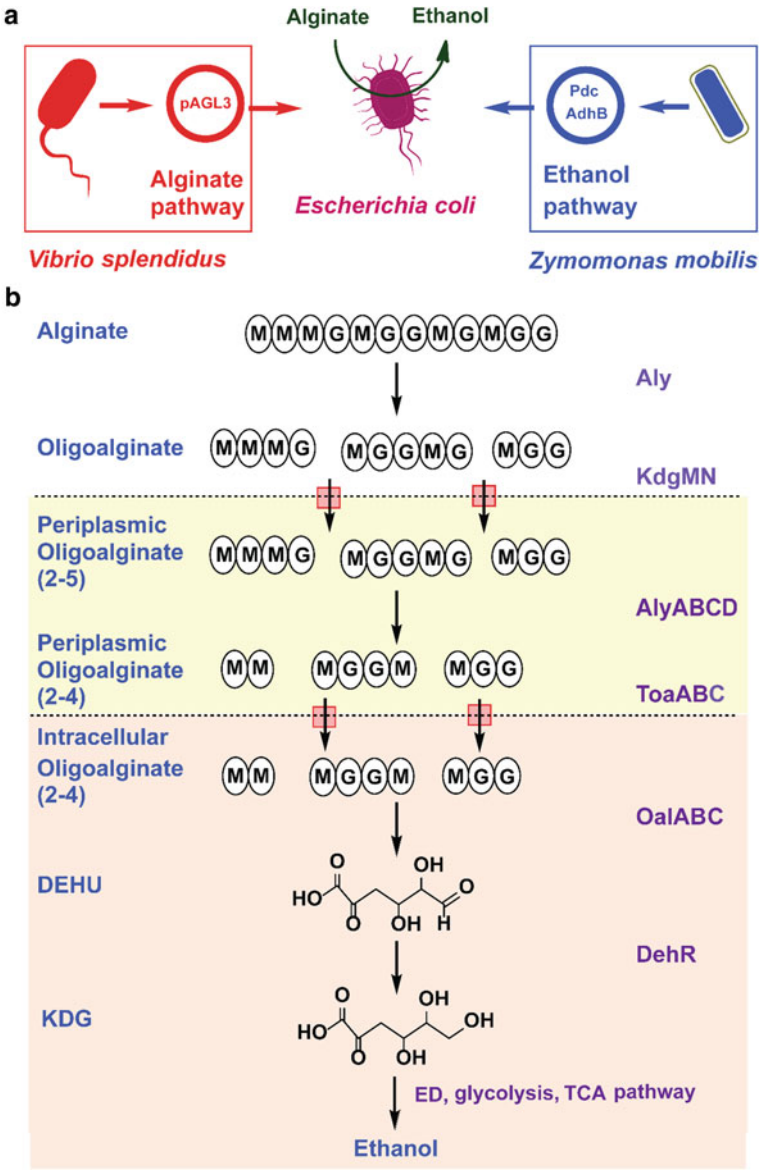

Fig. 5.5 *Escherichia coli* platform for the production of ethanol from macroalgae. **a** The fosmid pALG1 contained the genes for alginate metabolism from *Sphingomonas* sp. strain Al, and homoethanol pathway consisting *Zymomonasmobilis* pyruvate decarboxylase (Pdc) and alcohol dehydrogenase B (AdhB). **b** Alginate is degraded into oligomers by an alginate lyase. The oligomers relocated through the outer-membrane porins (KDGMN) into the periplasm. Oligomers with a degree of polymerization (DP) more than 3 are degraded into smaller oligomers by periplasmic alginate lyases (AlyABCD). These oligomers are transported into the cytosol via oligoalginate transporters (ToaABC). Oligoalginate lyases (OalABC) then degrade oligomers into monomer units (DEH). DEH is converted by DEH reductase (DEHR) to KDG, which enters the ED pathway

enhanced the alginate utilization by engineered *E. coli* strain. The heterologous pathway containing of pyruvate decarboxylase (Pdc) and alcohol dehydrogenase B (AdhB) from *Zymomonas mobilis* transferred into the engineered *E. coli*. *Saccharina japonica* (kombu) and brown macroalgae used as a fermentation substrate. *E. coli* can naturally produce ethanol by assimilating mannitol and glucose. A wild type *E. coli* without the engineered alginate assimilation pathway has been reported to produce $\sim 10\ gL^{-1}$ ethanol after 150 h. An engineered microbial platform produced ethanol at a final titer of ~4.7% v/v and over 80% of the highest speculative yield from macroalgae fermentation (Wargacki et al. 2012).

5.10 Brown Macroalgae Utilization Pathway in Engineered Yeast

S. cerevisiae has been engineered to convert brown seaweed into ethanol (Enquist-Newman et al. 2013) (Fig. 5.6). The four major modifications were applied, involving the rebuilding of a bacterial alginate catabolic pathway, DEHU transporter integration, down regulation of an innate mannitol catabolic pathway, and redox balance conservation.

In the first modification, the multiple enzymes in DEHU catabolism are overexpressed and efficient genes were chromosomally incorporated into both DEHU transporter selection strain and ethanol making strains. In the second modification, RNA-seq and cDNA library-based approaches were used to find a gene coding the DEHU transporter. The resulting strain was able to cultivate on DEHU as the only sugar substrate. In the third modification, microarrays were performed for the evaluation of *S. cerevisiae* strains cultivated in glucose, raffinose, and mannitol. The native mannitol 2-dehydrogenases and mannitol transporters were overexpressed. *S. cerevisiae* growth on mannitol was optimized by using different gene combinations. In the last modification, the genes in alginate and mannitol degradation were chromosomally incorporated into a *S. cerevisiae*. The cofactor selection of DEHR was examined to upkeep higher ethanol production from brown seaweed (Enquist-Newman et al. 2013).

S. cerevisiae wild type strains Lalvin and Pasteur Red produced $\sim 10\ gL^{-1}$ ethanol during growth on $30\ gL^{-1}$ mannitol. Ethanol fermentation was performed by mimicking the sugars present in brown macrolagae. The 1:2 molar ratio of DEHU:mannitol at 6.5% (w/v) and 9.8% (w/v) overall sugars were used in fermentation experiments using engineered strains. Ethanol was competently formed in both cases, accomplishing titers of 4.6% (v/v) ($36.2\ gL^{-1}$) and 83% of the highest theoretic yield from consuming sugars was reported (Enquist-Newman et al. 2013).

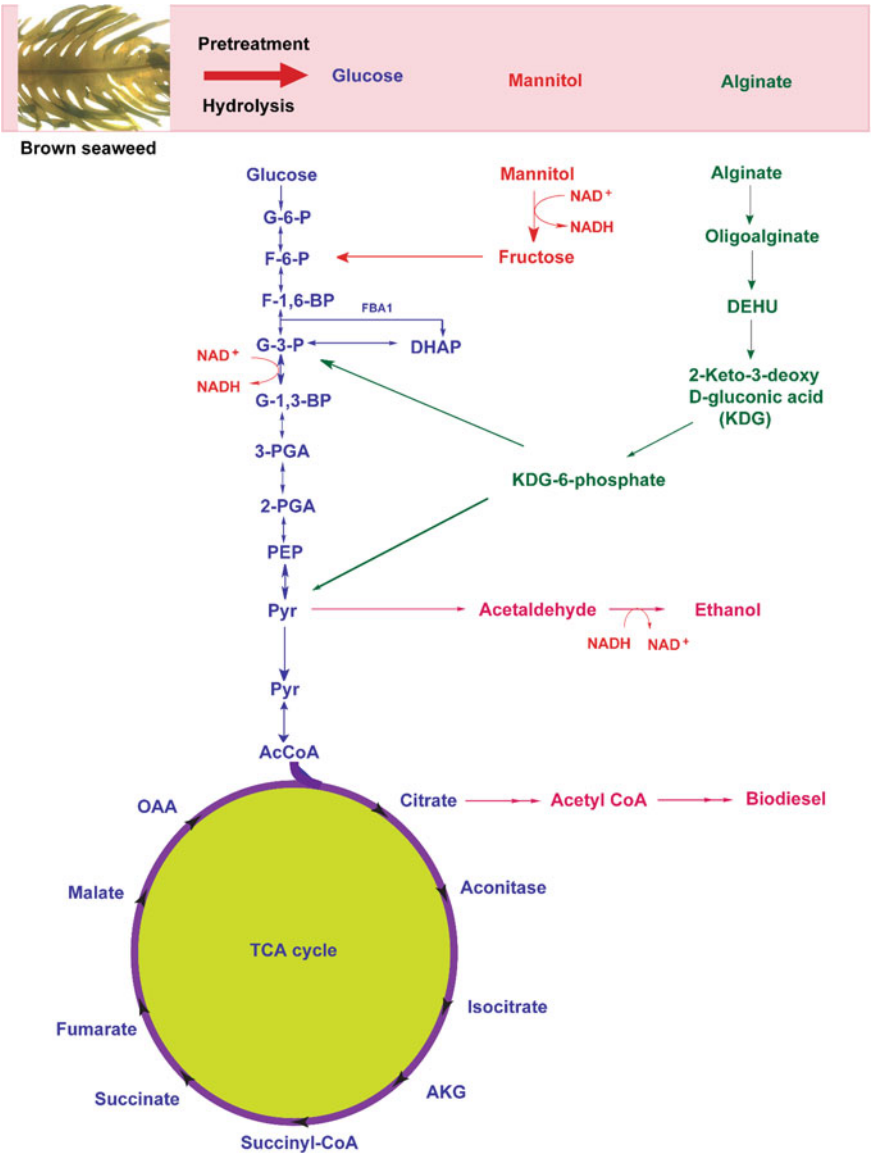

Fig. 5.6 *Saccharomyces cerevisiae* platform that can co-metabolize alginate, mannitol, and glucose to produce ethanol. The expression of heterologous alginate and optimization of endogenous mannitol pathways are essential to accomplish high ethanol production. Alginate is depolymerized into monomers by action of alginate lyases. DEHU is transported into the cytoplasm and converted into 2-keto-3-deoxy-D-gluconate (KDG) by DEHU reductase (DEHR). KDG is converted to the glycolytic intermediates

5.11 Products

Multiple microorganisms have been explored for the making of numerous value-enhanced target products from lignocellulosic sugars (Jagtap and Rao 2018a, b; Jagtap et al. 2019). The composition of lignocellulosic biomass and algal biomass is different (Bhatia et al. 2020). Macroalgal biomass has been explored for the manufacture of biogas, ethanol, and biodiesel. The different pretreatment methods are used including conversion using microbes, hydrothermal liquefaction (HTL), and pyrolysis. These technologies do not require macroalgae degrading enzymes, thus, they are not included here. The high carbohydrate content of macroalgae makes it a suitable substrate to produce biofuels including ethanol, 2,3-butanadiol, and 2,5-furandicarboxylic acid.

5.11.1 Bioethanol

Brown macroalgae is an ideal substrate for ethanol production. It is made up of a high percentage of carbohydrate and can be easily mass cultured using existing agriculture technologies. The certain microorganisms readily utilize mannitol and laminarin. Mannitol and laminarin extract from *L. hyperborea* were used to produce bioethanol (Horn et al. 2000a, b). *Pichia angophorae* concurrently consumed both mannitol and laminaran to produce ethanol. This yeast achieved the highest yield of 0.43 g ethanol g substrate. The enzymatic saccharification extract of *Saccharina latissimi* was fermented to ethanol and resulted in 0.45% (v/v) ethanol yield (Adams et al. 2009). *Pichia* yeast fermented mannitol, laminarin, and glucose into ethanol with a yield 0.43 g ethanol g substrate (Horn et al. 2000b). The extract of *Laminaria digitata* was fermented by *P. angophorae* and accomplished ethanol yield to 0.89% (v/v). An engineered *E. coli* KO11 metabolized mannitol and glucose. The concurrent saccharification and fermentation of the acid lysates of *S. japonica* resulted in a final ethanol yield of up to 29 gL^{-1} (Takeda et al. 2011).

E. coli platform was constructed to produce ethanol. The resulting strain BAL1611 made 37 gL^{-1} of ethanol or titer of ~4.7% v/v of ethanol from *S. japonica* extracts. Yeast platform is also developed for the production of ethanol. The concurrent consumption of DEHU and mannitol resulted in ethanol titers of 4.6% (v/v) using *S. cerevisiae* (Enquist-Newman et al. 2013).

5.11.2 Biobutanol

The acetone-butanol fermentation by *Clostridium* sp. resulted in biobutanol production from macroalgae (Kudahettige-Nilsson et al. 2015). *Clostridium* sp. can efficiently produce biofuels, and organic acids using various substrates. Contrarily,

this bacterium did not efficiently consume some glucose-based polysaccharides, resulted in slow reaction and productivity (Kudahettige-Nilsson et al. 2015).

Macroalgae can also be used as a substrate for the production of valuable chemicals. 2,3-butanediol and acetoin were produced using brown macroalgae. An engineered *E.coli* strain yielded 0.43 g/g of 2,3 butanediol (Mazumdar et al. 2013) The synthetic 2,3 butanediol pathway from *Enterobactor aerogenes* KCTC 2190 was integrated in *E.coli* strain. A commercial enzyme mixture was used for the *S. japonica* lysates pretreatment which was later fermented with engineered *E. coli* for 2,3-butanol production.

5.11.3 2,5-Furandicarboxylic Acid

2,5-furandicarboxylic acid (FDCA) can be a possible a precursor for polyethylene terephthalate (PET), nylons, and jet fuels production (Yoshikuni et al. 2016). Alginate degrading enzymes have been used to make DEH, which can subsequently convert into FDCA.

5.11.4 Methane

Sugars from macroalgae are converted to acetate, CO_2, and H_2 by microbial processes like acidogenesis and acetogenesis (Milledge et al. 2019; Giri et al. 2020; Sharma et al. 2020). Thereafter, it converted to methane and CO_2 by methanogens. Mechanically pretreated macroalgae such as *U. lactuca, Laminaria* spp., and *L. digitata* were incubated with cattle manure in bottles with rubber stoppers for 3–4 weeks. Methane yields were in the range of 150–330 mL g^{-1} volatile solids. The 34% higher methane yield was observed when washed and dried *U. lactuca* used as compared to that of unwashed and wilted (Milledge et al. 2019). Methane yield was 143–244 mL g^{-1} volatile solids in thermally pretreated macroalgae. Thermochemical pretreatment of *Ulva* spp., *F. vesiculosus*, and *L. digitata* enhanced methane yields by 2.5-fold as compared to untreated macroalgae (Milledge et al. 2019).

5.12 Conclusion

Macroalgae have many environmental and economic benefits and can be bulk cultured with current agricultural knowledge to generate biochemical and biofuels. The exploration of varying composition of carbohydrate in macroalgae is the future research needs to maximize their utilization and applications. The degradation of carbohydrates to release sugar requires specific enzymes and conversion of these

sugars into fuels and chemicals needs specialized metabolic pathways. These specific metabolic enzymes and pathways are not widely distributed in native microorganisms. These microorganisms are unable to depolymerize polysaccharides because of the absence of assimilation pathways. Few studies described the agarose and alginate metabolism in some microorganisms. However, more research is required to identify possible metabolism pathways of different polysaccharides for maximum utilization of the algal polysaccharides. Hence, the identification and characterization of unique polysaccharide converting enzymes and pathways are required for the development of efficient seaweed biomass degrading microbes. Metabolic engineering and synthetic biology approaches can become a new direction of research for the development of fermenting microorganisms that transform seaweed biomass to biofuels.

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Chapter 6
Seaweed-Based Biodegradable Biopolymers, Composite, and Blends with Applications

Sachin V. Otari and Jyoti P. Jadhav

Abstract The rising environmental awareness is promoting researchers to develop new biodegradable material. Seaweed is a versatile organism able to produce diverse type of polymers, i.e., agar, carrageenan, alginate, and polyhydroxyalkanoates. Several studies confirmed that these biopolymers are non-toxic, biodegradable, renewable, biocompatible, and eco-friendly. Seaweeds have been used as reinforcement to improve the mechanical properties of polymer composites. Several modifications have been investigated in the seaweed biopolymer to change or improve the properties of biopolymers like functionalization, blending with different polymers, and forming composite with supporting materials. The potential of seaweed as filler in polymer composites improves the thermal, physical, and mechanical properties of the synthetic polymer matrix. The chapter focuses on various seaweed biopolymers with their potential sources, modification of biopolymer, and their application in various fields.

6.1 Introduction

Plastic is human's most utilized product, which is moisture resistant, flexible, strong, and most importantly cheaper than any other product. Due to these properties of plastic, more than five decades, the consumption and production have increased by years. In 2008, the global consumption of the plastic was around 260 million ton which was increased to round 400 million ton by the end of year 2015. Plastic has made its potential contribution in medicine, space programs, transportation, and lifesaving equipment like incubators, helmets, ventilators, and most commonly the carriers for clean drinking water. The durability and slow or non-biodegradability property of plastic has given it more power to stay in the

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environment for very long time causing the most durable waste over other form of wastes. The constant demand, over consumption, mismanagement, and littering of the plastic gained lethal nature. The synthetic polymers like polyethylene (PE), polypropylene (PP), polyvinyl chloride, polyethylene terephthalate (PET), polystyrene, polycarbonate, and poly (methyl methacrylate) can be molded by heat and pressure which is further designated as plastic (Andreeßen and Steinbüchel 2019). Plastic is composed of very toxic chemicals, so accumulation in land and water causes severe environmental damage. Most plastic is produced from petrochemical resources which largely contribute in green house emissions. As plastic is made for durability, its non-degradability has created negative impact on each part of the ecosystem, killing plant life and posing dangers to local animals and humans.

To reduce the devastating impacts of plastic on ecosystem, the use of polymers derived from the biodegradable resources was advocated by scientific community. The biodegradable polymer or bioderived polymers are designated as biopolymers which is most innovative form of plastic produced from the renewable and degradable resources. So, to make the products ecofriendly, biodegradable, research was focused on incorporation of these biopolymers in natural or synthetic polymers such as starch and cellulose (Thakur et al. 2014; Appelqvist and Debet 1997). Cellulose is the most abundant biopolymer found in nature as it is the main component of the plant biomass. It is also major constituent of the bacterial extracellular polysaccharides (Limoli et al. 2015). So there are several attempts have been made to use cellulose fibers or cellulose fiber composite to replace the synthetic fibers in several clinical and industrial applications (Hickey and Pelling 2019; Abdul Khalil et al. 2012). Like cellulose, starch, gluten, guar gum, and chitosan are the most used natural biopolymers for several industries; food industry involves the edible films made up of starch for wrapping fruits and vegetables to increase their shelf life. The environmental and socioeconomic issues demand for the adaption of the technologies which can accommodate the bioderived polymers and their derivatives to reduce the fatal ecological impact of non-degradable plastic. In the year 2013, biopolymers production was 5.2 million ton contributing about 2% shares of overall polymer production. It is expected that by the year 2020 the production of biopolymer will be 17 million ton which may be 4% of overall polymer production. Marine algae are another abundant source of biodegradable polymers like proteins, nucleic acid, and polysaccharides. Edible seaweeds are form of marine algae which have been utilized as a source of food in many countries due to nutritious values. In addition, these seaweeds are potential and cheap source of biopolymers, e.g., polysaccharides-based polymers.

Seaweeds or marine macroalgae are plant-like edible form of microorganisms which are mostly found on the hard substrata or rock in coastal regions which are traditionally harvested and consumed by human for years. These macroalgae can be cultivated throughout the year as these can be grown in wide range of environment. Seaweeds are classified into three groups: red (*Rhodophyta*), brown (*Phaeophyta*), and green (*Chlorophyta*) seaweeds. The red and brown seaweeds are exclusively found in ocean. whereas freshwater is the habitat of green alga. Seaweeds contain concentration of minerals and vitamins contributing major source of healthier food

product for health benefits. These (red and brown) seaweeds are consumed in raw salads, soups, meals, cookies, etc. The seaweeds are chemically composed of the proteins, carbohydrates, lipids, vitamin, polyphenols, carotenoids, and micronutrients which vary with species. These seaweeds have been large source of carbohydrates as hydrocolloids like agar, alginate, carrageenan, and fucoidan which are applied in food industry, microbiology, biotechnology, and healthcare industries. The hydrocolloids from seaweeds form viscous dispersion or gels or films when dissolved in water due to a long chain of hydrophilic polymers. So, they are commonly used as thickening or gelling agents to regulate the properties of aqueous solutions. This chapter gives brief insight into the recent advances in seaweed-based polymers and their composite blends for potential applications in various fields.

6.2 Types of Seaweed-Based Biopolymers

6.2.1 Polysaccharides-Based Polymers

The seaweeds are abundance source of polysaccharide which gained tremendous attention due to their renewability, non-toxicity, biodegradability, and species specificity. The marine algae-derived carbohydrates are agar, alginates, carrageenan, fucoidan, and ulvan. For the last three decades, the cultivation of seaweeds and extraction of polysaccharides have gained immense importance due to extraordinary applications of polysaccharides in pharmaceutical, food, and healthcare industries.

6.2.1.1 Agar

The marine red algae cell wall from the family Gracilariaceae, Gelidiaceae, Pterocladiaceae, and Gelidiellaceae consists of agar as abundant form of polysaccharide. The function of the agar and other polysaccharides is to provide mechanical strength to the cell wall in algal cells similar to that of hemicellulose of the higher plants (Lechat et al. 2000; Domozych 2015). In addition to this, it also provides flexibility to the algal cells to tolerate the strong ocean waves and currents (Ficko-Blean et al. 2015; BeMiller 1996). The cell wall of the algae also provides the resistance to pathogens, high salinity, and extreme fluctuation in pH and temperature with desiccation (Lahaye and Rochas 1991; Percival 1979; Deniaud-Bouët et al. 2014).

Agar has a sugar skeleton consisting of two alternating polysaccharides: agarose and agaropectin (Fig. 5.1). The major sources of agar are extracts of *Gelidium*, *Pterocladia*, and *Gracilaria* species where the structural variation occurs with a pattern of sulfation of the repeating units of agar, i.e., D and L-galactose (Fig. 5.1). In case of *Gracilaria* sp., the sulfation at C6 of L-galactose through enzymatic

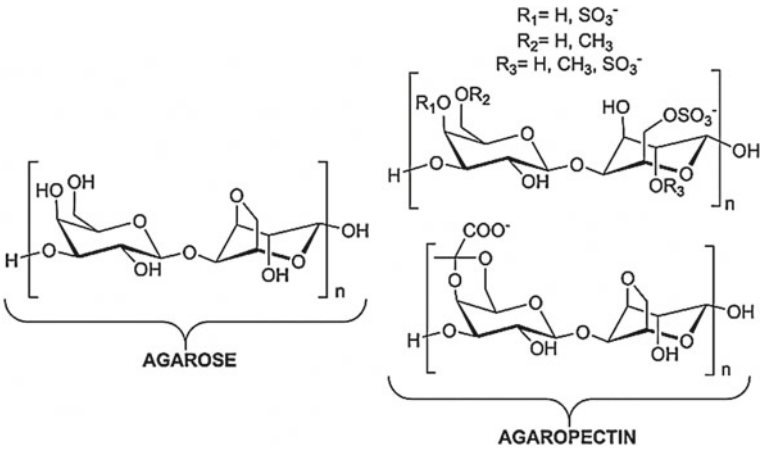

Fig. 6.1 Chemical structure of agar containing agarose and repeating disaccharide unit of agaropectin with different substituents [Reprinted with permission from Bertasa et al. (2017). Copyright 2017 Elsevier]

reaction using 3,6-anhydro-l-galactose as precursor occurs inadequate compared to that of *Gelidium* sp. and *Pterocladia* sp. which gives high degree of sulfation in the agar extracted from *Gelidium* and *Pterocladia* sp. Due to low sulfation reaction, the agar obtained from *Gracilaria* sp. has weak bonding during gelling procedure giving no gel or weak gel (Murano 1995). The chemical structure of the agar is essential to define physical properties of the extracted agar in terms of melting temperature, gelling and gel strength. With the alkali treatment before the extraction of agar from *Gracilaria* sp., the gelling properties of agar can be improved (Murano 1995). With a helical confirmation of polysaccharides of agar, gelation process generally occurs which is further aggregated through hydrogen bonds. The important feature of the agar gel is its thermo-reversibility. A temperature over 85 °C is required to melt the agar which forms gel upon cooling. The temperature requirement to melt and form gel is exclusively based on algal species as the gelling temperature depends on the methoxy group present in agar which varies with algal species (Guiseley 1970). The extent of methoxylation of agar polymer from *Gracilaria* sp. is higher compared to that of in *Gelidium* and *Pterocladia* sp. So, gelling temperature of agar from *Gracilaria* sp. is in the range of 40–45 °C which is much lower (34–36 °C) in case of *Gelidiaceae* sp. The gelling properties of the agar provide the opportunity to use biopolymer in wide range of applications in the field of pharmaceuticals, biomedical, environmental, biotechnology, and cosmetics. The worldwide market for agar is expected to grow at a CAGR of roughly 3.9% over the next five years, which will reach 350 million US$ in 2024, from 280 million US$ in 2019, according to a new study. Nevertheless, despite its degradability and its excellent gelling power, agar has not been used extensively due to its poor aging properties. The agar polymer structure gets ruptured or causes polymer

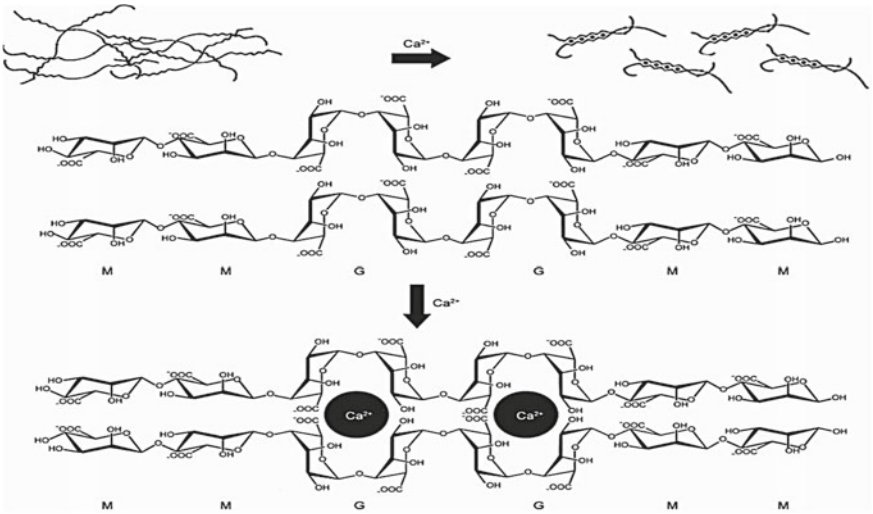

Fig. 6.2 Gelling mechanism of alginate in the presence of Ca^{2+} ions [Reprinted with permission from Tavassoli-Kafrani et al. (2016). Copyright 2016 eXPRESS Polymer Letters]

embrittlement due to photodegradation and fluctuations in ambient temperature and humidity, altering the agar's crystallinity (Shit and Shah 2014; Faridi Esfanjani and Jafari 2016).

6.2.1.2 Alginate

Like cellulose, the alginates are the most abundant polysaccharides derived from brown seaweed wall, *Macrocystis pyrifera*, *Laminaria hyperborea*, and *Ascophyllum nodosum* with several bacterial strains like *Azotobacter* and *Pseudomonas* (Sachan et al. 2009; Remminghorst and Rehm 2006; Rehm 2010). In *Ascophyllum nodosum*, alginates constitute about 22–30%, whereas in *Laminaria hyperborea* it constitutes about 25–44% of the dry algal biomass (Qin 2008). Alginate is composed of a linear polysaccharide copolymer of β-D-mannuronic acid (M) and α-L-guluronic acid (G) linked with 1,4-glycosidic linkage (Fig. 5.1). Alginate can be consisted of three forms of segments or blocks: homopolymers of MM and GG and alternating MG. The basic property of alginate largely depends on the concentration of M and G with distribution of the block throughout the molecule. In the presence of divalent metals, the alginate can form heat stable gel (Fig. 5.2). The physical and mechanical properties of the gel vastly depend on the three blocks and the M/G ratio in alginate which varies with species to species. The high G content gives stronger gelling property to alginate, whereas the higher viscosity is due to high M content in the alginate (Hernández-Carmona et al. 2013).

Alginates have four essential properties. First one is that alginates increase the viscosity of the water when dissolved in water. The second is their ability to form gels in divalent metal solution. The sodium salt is replaced by the calcium ions once sodium alginate is dissolved in the calcium ion containing solution. There is requirement of heat to make gel, and once the gel is formed, it does not melt on heating. The gel can be modulated by adjusting the salt concentration or the pH of the surrounding medium. The third property is the ability to form the calcium alginate film or fiber. And it is non-toxic, biocompatible, biodegradable, and inexpensive for production which is its fourth property. Considering all these properties, the hydrogel, nanoparticles, or fiber based on the alginates has been explored for several pharmacological, clinical, biomedical, biotechnological, and environmental applications.

6.2.1.3 Agarose

Agarose is a polysaccharide derived from the agarocytes of *Rhodophyceae*, and a class of marine red seaweed predominately grows in the Indian and Pacific oceans. As it is a neutral, water-soluble polysaccharide, it is explored for biomaterial (protein, enzymes, bacterial, mammalian cells) encapsulation applications (Iwata et al. 1992). Agarose is a major constituent of the agar polysaccharide contributing more about 70% in the structure. Agarose is a linear polymer with a molecular weight of about 120,000, consisting of alternating D-galactose and 3,6-anhydro-L-galactopyranose linked by α-(1 → 3) and β-(1 → 4) glycosidic bonds (Fig. 5.3) (Marinho-Soriano and Bourret 2005; Bertasa et al. 2017). The 3,6-anhydro-L-galactopyranose is an L-galactose with an anhydro bridge between the three and six positions, and the bridge may be absent in some L-galactose. Some D-galactose and L-galactose units can be methylated, and pyruvate and sulfate are also found in small quantities. About 800 molecules of galactose containing agarose chain form helical fibers which transform into supercoiled structure on aggregation. The length of the fibers depends on the concentration of agarose. After solidification, the agarose fibers form a three-dimensional network of channels of diameter in 50–200 nm range depending on agarose concentration; higher the concentration lowers the pore diameter.

Agarose is a preferred matrix for work with proteins and nucleic acids as it has a broad range of physical, chemical, and thermal stability, and its lower degree of chemical complexity also makes it less likely to interact with biomolecules.

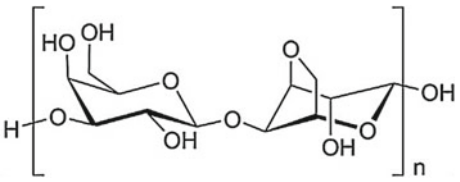

Fig. 6.3 Structure of the repeating unit of an agarose polymer [Reprinted with permission from Bertasa et al. (2017). Copyright 2017 Elesvier]

Agarose is most commonly used as the medium for analytical scale electrophoretic separation in agarose gel electrophoresis. Recently, agarose fiber, hydrogel, or its blends have been extensively studied for the biomedical applications like drug delivery, wound dressing materials, and scaffold for stem cell growth (Zarrintaj et al. 2018; Marras-Marquez et al. 2014).

6.2.1.4 Carrageenan

Carrageenan is another form of sulfonated polysaccharide present in the red seaweed which is extracted using dilute alkaline solutions. Though it is composed of partial sulfonated galactans, it is water-soluble polysaccharide forming very viscous solution which is stable at wide pH range. The few forms of carrageenans are soluble in cold water, whereas some are soluble in hot water forming a gel upon cooling and cross-linking in the presence of calcium or potassium ions. These varied properties are due to the degree and pattern of sulfation of polysaccharide. It is composed of alternate units of D-galactose and 3,6-anhydro-galactose (3,6-AG) joined by α-1,3 and β-1,4-glycosidic linkage (Fig. 5.4). There are three types of carrageenan possessing different chemical structures, contributing to different gelling properties (Necas and Bartosikova 2013; Blakemore and Harpell 2009); kappa (κ), lambda (λ), and iota (ι) each with its own gelling property. *Eucheuma denticulatum* is the source of iota-carrageenan and kappa-carrageenan form can be obtained from *Kappaphycus alvarezii* (Rasmussen and Morrissey 2007). A stronger gel can be obtained from kappa-carrageenan form with potassium and calcium ion as cross-linking ions in which rigid and elastic nature is provided by potassium ions, whereas calcium ions give a stiff and brittle property to the gel. To obtain rigid, elastic, and strong gel, iota and lambada form of carrageenans can be blended

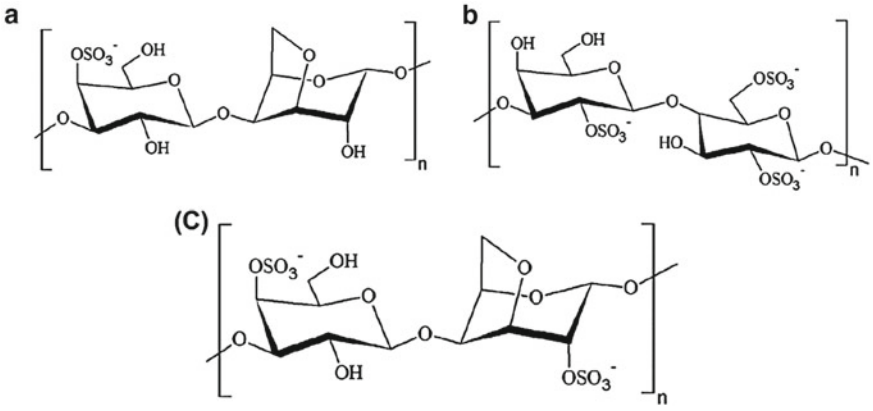

Fig. 6.4 Structure of κ-carrageenan (**a**), λ-carrageenan (**b**), and ι-carrageenan (**c**) [Reprinted with permission from Cunha and Grenha (2016). Copyright 2016 MDPI]

to form composite polymer. The antiviral and antitumor activities of the carrageenans are demonstrated in various reports (Skoler-Karpoff et al. 2008; Vlieghe et al. 2002). It is also used as stabilizing material in several medicinal creams and lotions. They are also used in internal poultices for the treatment in stomach ulcers. In some bowel movement-related cases like dysentery, diarrhea, and constipation, carrageenans showed its efficacy in treating these medical issues (Li et al. 2014).

6.2.1.5 Ulvan

Ulvan is a complex acidic sulfated polysaccharide extracted from the cell walls of the green seaweed Ulvales (Chlorophyta) (Chiellini and Morelli 2011). The two main species of *Ulva* and *Enteromorpha,* mainly cultivated for food consumption or other associated with proliferation at eutrophicated coastal waters leading up to hypoxia and death of most of aquatic organisms (Morand and Briand 1996), are considered as major source of ulvan like polysaccharide-based biopolymers. Around 35–48% of the cell wall polysaccharide constitutes the dry cell mass of these algae (Lahaye and Kaeffer 1997) which are ulvan, cellulose, linear xyloglucan, and a glucuronan. The major polysaccharide, i.e., ulvan extracted from the algal cell wall constitutes approximately 8–29% of the dry algal cell weight depending on the extraction and purification steps (Lahaye et al. 1997; Abdel-Fattah and Edrees 1972; Lahaye et al. 1994). Lahaye et al. extensively investigated the composition analysis of the ulvan carbohydrate demonstrating the very complex and variable composition containing numerous oligosaccharides in natural or chemically modified form in ulvan preparations (Lahaye and Robic 2007). In the ulvan structure, sugars like rhamnose, xylose, glucuronic, and iduronic acid with sulfate groups are the main constituents of the polymer. The main repeating disaccharide units reported are ulvanobiouronic acid 3-sulfate types containing either glucuronic or iduronic acid. In addition, minor repeating units have been reported to contain sulfated xylose, replacing the uronic acid or glucuronic. The methodological, taxonomic, and/or ecophysiological origins of the algal species may be the reason for the sugar composition variation. The arrangement of these monomers in the most extracted ulvan polysaccharide is linear but the

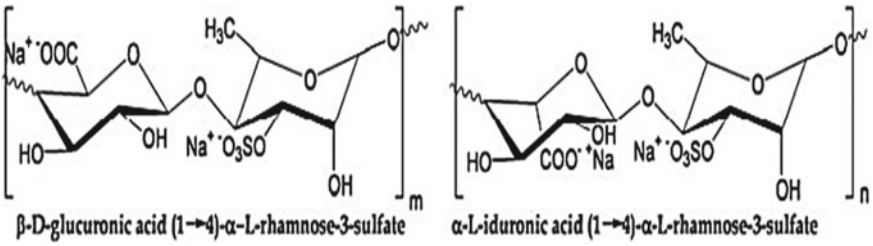

Fig. 6.5 Structure of the main disaccharide repeating units in ulvan [Reprinted with permission from Morelli et al. (2016). Copyright 2016 Elsevier]

slight branching in the structure was also observed (Lahaye and Robic 2007). The presence of the repeating dimeric sequence of aldobiuronic acid disaccharide such as type A (glucurorhamnose 3-sulfate, A3s) and type B (iduronorhamnose 3-sulfate, B3s) within the heteropolymer chain contributes the chemical heterogenicity to ulvan (Morelli et al. 2016) (Fig. 5.5).

Ulvan is water soluble due to its highly charged structure and hydrophilic nature. The dissolved ulvan does not show clear and transparent solution indicating formation of aggregates in the water which was confirmed by transmission electron microscopy (TEM) studies, representing its poor gelling property (Robic et al. 2009). The presence of large methyl groups provided by sulfated rhamnose in the polymer is responsible for the formation of unusual aggregates in the water which limits its use in biomedical application where the water acts solvent. Also, the functional group modification is difficult due to its insolubility in organic solvent (Robic et al. 2009). The biological activity of ulvan was extensively studied both in vitro and in vivo to demonstrate its antioxidant, anticoagulant, antihyperlipidemic, immunomodulating, and antitumor activities (Zhang et al. 2008; Qi et al. 2006; Leiro et al. 2007; Chiellini and Morelli 2011; Pengzhan et al. 2003). A material suitable for biomedical applications, namely tissue engineering, regenerative medicine, and drug delivery, is required to be biocompatible and biodegradable and its products of degradation must be safe and easily cleared from the host organisms. To use ulvan-based biopolymers or biomaterials in biomedical applications, ulvan needs to convert to water-insoluble form under physiological conditions and to have mechanical strength appropriate blends must be form. The UV-mediated radical polymerization of ulvan conjugating with macomers by double bond showed promising preparation of stable and strong cross-linked ulvan hydrogel which is providing attractive platform for the ulvan-based biopolymers to be applied in biomedical applications.

6.2.1.6 Fucoidan

Fucoidan is principally composed sulfated L-fucose belonging to a group of polysaccharides fucans. Other polysaccharides like arabinose, xylose, mannose, glucose, and galactose contribute less than 10% of them. Fucoidan is present in only cell wall of brown seaweed, mozuku sea algae (*Nemacystus decipiens*), kombu kelp (*Laminaria japonica*), wakame seaweed (*Undaria pinnatifida*) and hijiki seaweed (*Sargassum fusiforme*) (Berteau and Mulloy 2003). The major role of fucoidan in algal cell wall is to give strength and protect cells from the pathogen. Under dry conditions, it also prevents the desiccation of the algal mass. It is soluble in water and gives viscosity to the water at very low concentration. Algal fucoidan possesses one type homofucose backbones out of two types. The (1 → 3)-linked a-L-fucopyranosyl residues or alternating (1 → 3) and (1 → 4)-linked a-L-fucopyranosyl residues present in algal fucoidan. Algal species, e.g., *Cladosiphon okamuranus*, Laminaria digitate, and *Chorda filum* possess first type

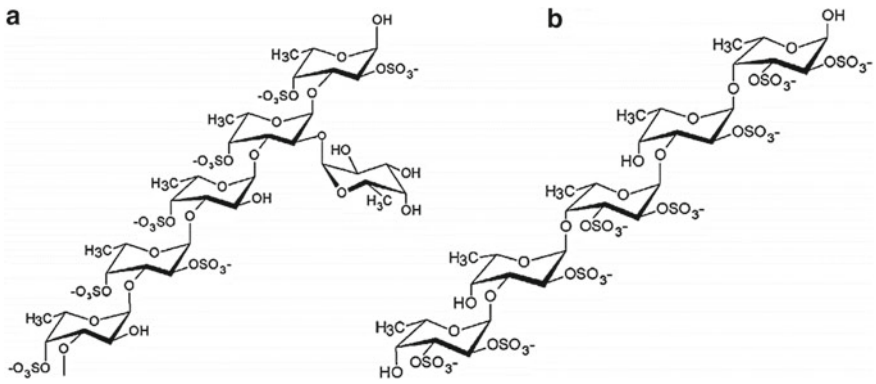

Fig. 6.6 Fucoidan structure from *Chorda filum* (**a**) and oligofucoidan from *Cladosiphon okamuranus* (**b**) [Reprinted with permission from Ale and Meyer (2013). Copyright 2013 Royal Society of Chemistry]

of fucoidan backbone, whereas the second type fucoidan backbone molecule is present in *Ascophyllum nodosum, Fucus distichus, and Fucus evanescence* (Fig. 5.6)

Fucoidan has been explored for its vital application in clinical and pharmaceutical applications due to its antiviral, anti-inflammatory, anticoagulant, antioxidant, and immunomodulator activity (Li et al. 2008). The functional properties of fucoidan have provided the additional advantage to be a part of biomaterials which are applied for drug delivery, wound healing, bone regeneration composites, and in vivo scaffolds for stem cell proliferation. Ho et al. used fucoidan from two algal species *Fucus vesiculosus* and *Undaria pinnatifida* for preparation of the film and studied extensively for structural variation (Ho et al. 2015). Fucoidan has been demonstrated for the heparin like activity modulating the effect of growth factors. The composite film of fucoidan and chitosan promotes contraction and re-epithelization of wounds, proving its possible application in wound healing in hydrogel form in burn situations (Sezer et al. 2007). Few more examples of carrageenan, fucoidan, ulvan polysaccharides derived from three main species of seaweeds (red, brown, and green algae) with their monosaccharide composition, molecular weight, backbone, and structure function are summarized in Table 5.1.

6.2.2 Polyhydroxyalkanoates

Currently, the vast research is going to produce the material which is like plastic. The polyhydroxyalkanoates (PHA) are another diverse form of bioderived polymers produced from varied gram positive and gram negative microorganisms which are a storage granules in the microbial cell under environmental stress conditions (Reddy et al. 2003). Among the various biopolymers used for different applications,

Table 6.1 Examples of carrageenan, fucoidan, ulvan polysaccharides derived from three main species of seaweeds (red, brown, and green algae) with their monosaccharide composition, molecular weight, backbone, and structure function

Species	Polysaccharide type	Molecular weight (Da)	Monosaccharide	Backbone	Biological activities	References
Red algae						
Mastocarpus stellatus	Carrageenan	1248 kg	Gal:Glc:Xyl: Man = 87.8:5.4:4.4:2.4	β-1,3-Gal and α-1,4-Gal	Anticoagulant	Youssouf et al. (2017)
Chondrus armatus	Carrageenan	88 kg	Gal	β-1,3-Gal and α-1,4-Gal	Antiviral	Kalitnik et al. (2013)
Hypnea musciformis	Carrageenan	6–788 kg	Gal	3-β-d-galactose-4-sufhate and 4-3,6-anhydro-α-d-galactose	Antimicrobial Antioxidant	Souza et al. (2018)
Brown algae						
Cystoseira sedoides	Fucoidan	642 kg	Fuc and Uronic acid	α-1,3 or α-1,4-Fuc	Anti-inflammatory	Ammar et al. (2015)
Coccophora langsdorfii	Fucoidan	–	Fuc	α-1,3 and α-1,4-Fuc	Anticancer	Imbs et al. (2016)
Undaria pinnatifida	Fucoidan	100–300 kg	Fuc and Gal	1,3-linked fucose, and 1,3-, 1,4-, and 1,6-linked galactose	Antiviral	Lee et al. (2004)
Green algae						
Capsosiphon fulvescens	Ulvan	–	Rha:Xyl: Man = 45.0:44.1:10.2	4)-β-Xyl-(1 → 4)-α-Rha-(1→	Anticoagulant	Synytsya et al. (2015)
Ulva armoricana	Ulvan	140–500 kg	Rha:Gal:Glc: Xyl = 40.0:6.7:26.2:4.4	–	Antiviral	Hardouin et al. (2016)
Ulva pertusa	Ulvan	28.2 kg	–	–	Antiradiation Antihyperlipidemic	Shi et al. (2013)
Ulva conglobata	Ulvan	–	Rha:Gal:Glc:Xyl:Man	→4)β-D-GlcA (1 → 4) α-L-Rha3S-(1→	Anticoagulant	Mao et al. (2006)

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Gal galactose; *Glc* glucose; *Xyl* xylose; *Man* mannose; *3,6-AnGal* 3,6-anhydro-D-galactose; *Ara* arabinose; *Fuc* fucose; *Rha*, rhamnose

PHA has been explored extensively due to its properties such as tunable mechanical strength, cytocompatibility, and biodegradability (Bhatia et al. 2019a, b). The number microorganisms have been reported for production of PHA and its derivatives (Reddy et al. 2003). The microorganisms utilize carbon source to form small chain linked copolymers of PHAs like poly (3-hydroxybutyrate-co-3-hydroxyvalerate) PHBV or poly (3-hydroxybutyrate-co-4-hydroxybutyrate) PHB4B (Patel 2012, 2015). The constituents of the block copolymers of PHAs can be modulated by altering the substrates and growth conditions. The microalgae, like cyanobacteria, are gram negative bacterial considered as blue-green algae, showed production of PHB under phototrophic conditions (Balaji et al. 2013). Several cyanobacterial species have been demonstrated for the accumulation of the PHB polymer. There are very few or no report which states the production of PHA in seaweeds. Ghosh et al. used microalga (*Ulva* sp.) hydrolysate as a carbon source to produce PHA by *Haloferax mediterranei* (Ghosh et al. 2019).

6.3 Blend Polymers

The polymers obtained from the seaweeds are biocompatible, non-toxic, renewable, gelling property, and scope for chemical modifications. The hydrogels or films formed using seaweed polymers have very poor mechanical properties like flexibility, high hydrophilicity, strength, and stability. So, seaweed polymer-based gels cannot be utilized for many industrial applications like food packaging and textile industries. The polymers blends using seaweed polymer and other biopolymers are prepared to increase the properties of the gels and films obtained from seaweed polymers. Several polymers which are non-toxic, biodegradable like polyvinyl alcohol (PVA), polyvinyl caprolactam (PVC), starch, carboxymethyl cellulose (CMC), gellan gum, gelatin, proteins, lipids, and various natural polymers are blended with seaweed polymers.

Madera-Santana et al. constructed the polymeric blend of agar and PVC and studied the various properties of agar, PVC, and agar-PVC films. They also extracted agar from the algal species in different seasons and compared their properties due to chemical changes occur in agar (Madera-Santana et al. 2011). Edible film based on agar, cassava starch (CAS) and arabinoxylan (AX) was prepared to study the microstructure, moisture barrier, and mechanical properties (Phan The et al. 2009). Mechanical properties of CAS-AX-based film were significantly improved by the addition of agar in the film. Blend of food hydrocolloids, viz. agar and kappa-carrageenan cross-linked with in aqueous medium forming the cross-linked blend with improved thermal stability, swelling ability, and higher viscosity (Meena et al. 2009). The different soy protein isolate and agar with different proportion have been blended to form film with improved water uptake ability, surface morphology, and weight loss profile (Rivadeneira et al. 2018). The efficient release of the antibiotic, ciprofloxacin hydrochloride, was observed showing efficient antimicrobial properties against *Pseudomonas aeruginosa*

species. The ternary blend of biohydrogel films agar/κ-carrageenan/konjac glucomannan improved mechanical and water vapor barrier properties (Rhim and Wang 2013). The ternary blend nanocomposite showed antimicrobial activity against *Listeria monocytogenes*. Felfel et al. prepared the polymeric blend of chitosan and agarose forming 3D scaffold forming hydrogen bonding between agarose and chitosan which provided strong mechanical support for repairing soft tissues (Felfel et al. 2019). Different proportions of the alginate and CMC were blended to form bilayer film for wound healing dressings where varying concentrations of blends allowed to formulate suitable wound dressing matrices (Trevisol et al. 2019). Several blends have been constructed with seaweed polymers to make them applicable in industries like food, textile, pharmaceutical, etc.

6.4 Applications of Seaweed Polymers

The seaweed-based polymers and the composite blends have been proved to be non-toxic and biocompatible to the biological entities. So, these biopolymers have been demonstrated for several biomedical, industrial, and remedial applications.

(a) **Biomedical applications**

(i) *Drug Delivery*

The design of the effective drug delivery system (DDS) is one the most essential steps for the site-specific delivery of pharmaceutical drugs or natural compounds to enhance the efficacy of the drugs and distribution of the drug in non-specific cells. So far, these DDS have been studied and commercialized using liposome, microspheres, gels, and prodrugs. Various biopolymers like chitosan, cellulose, starch, silk fibroins, collagens, and albumin have been explored for the delivery of drugs (Song et al. 2018). For the last decades, the seaweed-based biopolymers are being extensively studied due to their non-expensive, abundant availability, biocompatibility, non-toxicity, and most important biodegradability properties. The gelling phenomenon of the polysaccharide and their blends allow them to be part effective drug carrying hydrogels, microgels or nanogels.

Among the various forms of seaweed biopolymer, alginate is the most explored polysaccharide for the delivery of the bioactive molecules like peptide, proteins, cytokines, growth factors, and other drug molecules. There are several studies have been conducted to engineer the ideal DDS which deliver the desired drug at specific site increasing the permeability and stability of delivering vehicle or capsule. Matsusaki et al. used layer-by-layer assembly of the polymers to encapsulate the vascular endothelial growth factor (VEGF) where the alginate nanohydrogel layer was coated on the polyelectrolyte multilayer (PEM) films composed of chitosan (CT) and dextran sulfate (DEX) to maintain structural integrity of the VEGF carrying vesicle which was found to be collapsing without alginate hydrogel layer (Matsusaki et al. 2007). The modifications of carrying system to minimize the

inflammatory response of the host cells are also another task for effective delivery of the drug. The functionalization of the alginate layer in the DDS is with immunosuppressive drugs like antibodies (anti-TNF-α) (Leung et al. 2008) and heparin (Bünger et al. 2003) by physical adsorption. The several strategies have been employed to modify the drug carrying capsule which allows to administer the drug in various ways like oral, intravenous, etc. For instance, in oral drug administration, the DSS must escape from the gastrointestinal digestive system. So, investigators introduced several improvements to drug delivering vesicles having alginate as the outermost layer like introduction of glycoprotein layer through disulfide linkage (Greimel et al. 2007), covalent cross-linking with chitosan (Taqieddin and Amiji 2004), etc. Intravenous administration of alginate-based DSS is also another challenging task as where the delivering vesicles must circulate until it reaches to desired site. Sangeetha et al. treated systemic candidiasis in a murine model using alginate nanospheres containing amphotericin B which showed higher antifungal efficacy in comparison with the conventional formulation of amphotericin B (Sangeetha et al. 2007). Ahmad et al. used alginate nanoparticles encapsulating econazole and antitubercular drugs (ATDs) against murine tuberculosis (Ahmad et al. 2007). The polymeric blend of oxidized alginate with gelatin has been successfully demonstrated as injectable slow release vesicles for the drug primaquine (Balakrishnan et al. 2005). The hydrophobic modification through long chain alkyl of alginate was also demonstrated for the delivery of protein molecules at targeted site where 70–100% encapsulation of protein was observed in alginate nanoparticles (Leonard et al. 2004). Due to the high biocompatibility and biodegradability with low toxicity of alginate or alginate blends, these biopolymers have been the most favored biomaterials for several experiments of microencapsulations, immunoisolations, and xenotransplantation of cells where the commercialization of the biopolymer-based products is practiced (Grøndahl et al. 2019). The highly viscous suspension forming carrageenan has been commercialized as the hydrophilic matrix system for the sustainable (zero order) release of the encapsulated drug from the tablet. Two commercial products have carrageenan in the tablets, e.g., Gelcarin® GP-379 (ι-CG) and Viscarin® GP-209 (λ-CG) for the preparation of extended release tablets for tripelennamine HCl (model drug). Due to highly viscous nature of the carrageenan, it is difficult to obtain the controlled release and pH-independent drug release when only carrageenan-based matrix is prepared. Nerurkar et al. designed the tablets prepared from polymeric blend of carrageenan–cellulose ethers for sustainable release of ibuprofen where the sustained release was obtained for 12–16 h (Nerurkar et al. 2005). Baloglu and Senyigit formulated the two or three-layered tablets made up pectin, guar gum, xanthan gum, CS, and ethyl cellulose, carrageenan for the sustained release of metoprolol tartrate where carrageenan and its blends were found to be most effective compared to other swollen polymers exhibiting super case II release mechanisms (Baloğlu and Şenyiğit 2010). The viscosity of carrageenan gel can be controlled by varying pH of the surrounding medium. A double-layered polyelectrolyte complexes microbead was constructed using alginate, chitosan, and κ-carrageenan for the release of 5-fluorouracil in gastrointestinal condition which is

pH-responsive and could be managed by varying the polymer concentrations (Sun et al. 2019). The carrageenan forms the strong interactions with positively charged molecules due to the presence of the half-sulfated moieties in the structure. Bonferoni et al. prepared in situ polyelectrolyte complex formation between diltiazem (basic drug) and carrageenan (λ form) in distilled water for controlled release in pH 1.2 and 6.8 which showed complete release of the drug in one hour (Bonferoni et al. 2004). Li et al. extensively reviewed various forms of the carrageenan biopolymers and their blends with other biopolymers to form desired drug carrying vesicles for sustained release at targeted sites (Li et al. 2014). Due to high gelling temperature, natural agarose-based polymers or hydrogels have very limited applications in the field of drug delivery. Investigators modified the agarose by introducing functional through acylation, oxyalkylation, alkylation, alkenylation, and acetylation which lowers the gelling temperature of agarose hindering the formation of helical structure at low temperature (Kim et al. 2019; Zhang et al. 2008). Kim et al. functionalized agarose with β-cyclodextrin lowering the gelling temperature of the agarose allowing to encapsulate the bovine serum albumin and doxorubicin in the hydrogel structure which was further demonstrated for the anticancer activity (Kim et al. 2019). The agarose hydrogels were also modified with anionic or non-ionic surfactants which modified the pore structure of the hydrogel allowing control over the release of drugs having different solubility (Marras-Marquez et al. 2014). The polymeric blend of agarose and alginate was designed to form hydrogel carrying ciprofloxacin and selenium modified borosilicate glass nanoparticles to apply as the antibacterial drug delivering hydrogel (El-Kady et al. 2020). Ulvan and fucoidan are the two unexploited seaweed-based polymers for drug delivery applications as alginate was having the major focus among all other seaweed biopolymers. There are few studies that have been conducted in polymeric blends of ulvan and fucoidan polymers. Alves et al. designed 2D and 3D membrane structure by cross-linking with the epoxide 1,4-butanediol diglycidyl ether (BDDE) for drug delivery application in wound management (Alves et al. 2012b, 2013). The electrospinning technique was employed for the construction of the water-soluble polymeric blends of ulvan/polyvinyl alcohol (PVA), ulvan/polyethylene oxide (PEO), and ulvan/polycaprolctone (PCL) for possible application in drug delivery (Kikionis et al. 2015). Fucoidan-based polymers and nanoparticles are also demonstrated as the efficient carrier for drugs. Fucoidan-based particles are used as carriers for drugs, growth factors, proteins, genes, etc. (Citkowska et al. 2019). Here the fucoidan polymer not only acts as drug carriers but also is used as therapeutic agent as it can be used as effective immunomodulator. The colonic drug delivery was performed using cisplatin (CSN) and pure fucoidan which was biocompatible and non-cytotoxic (Hwang et al. 2017).

(ii) *Wound healing matrix*

Wound dressings are one of the most essential materials in the wound management which should not only be effective also it should be affordable. Wound dressings are mostly made up of biopolymers, biomaterials, and synthetic or natural

polymer. Currently, biopolymers like alginate, fucoidan, chitosan, hyaluronic acid, etc., are used for the dressing materials which are non-toxic, biocompatible, non-immunogenic, and biodegradable. Among all biopolymers, alginate has been studied extensively. There are several commercial alginates-based wound dressings, e.g., Pharma-Algi®, Pharmaplast, Nu-derm®, Algisite M®, Melgisorb®, etc., are available in the market. Due to the weak mechanical strength of seaweed-based polymers, these biopolymers are difficult to form stable structures. Straccia et al. demonstrated the alginate–chitosan hydrogel has potential to be effective wound dressing materials having antimicrobial activity against *Escherichia coli* (Straccia et al. 2015). The in vivo formation of new blood vessels and enhanced re-epithelialization of cutaneous wound was observed when wound was treated with simvastatin encapsulated in alginate hydrogel (Yu et al. 2016). The alginate fiber was blended with 2,2,6,6-tetramethylpiperidine-1-oxyloxidized bacterial cellulose (TOBC) to construct non-toxic biohydrogel which was further loaded with Zn^{2+} to provide antibacterial property to the biohydrogel (Zhang et al. 2020). The essential oils blue, cinnamon, lavender, tea tree, peppermint, eucalyptus, lemon grass, and lemon oils were incorporated in alginate film which showed selective inhibitory action against *Candida albicans* (Liakos et al. 2014). Chabala et al. constructed wound healing and antibacterial chitosan–alginate film by incorporating aloe vera gel and silver nanoparticles (Chabala et al. 2017). The dexpanthenol-loaded wound dressing biomaterial composed of PVA-alginate nanofibers which followed the Fickian diffusion mechanism where the composite nanofiber was not only non-toxic also the attachment of cells was also observed (Tamizi et al. 2018). The foam is another form of wound healing matrix that can be applied to the wounds which are lightweight and more effective. Hegge et al. designed curcumin loaded alginate foam which showed efficient antibacterial activity against *Enterococcus faecalis* cells in vitro (Hegge et al. 2011). Fucoidan-based polymers in wound dressing matrices provide significant advantages as fucoidan is not only biocompatible and biodegradable also it acts like immunomodulator enhancing the immune response of the host cells. The fucoidan–chitosan polymeric blend-based hydrogel showed enhanced burn healing property in rabbit model (Sezer et al. 2008). The polymeric blends of alginate, fucoidan, and chitin/chitosan were demonstrated for promising wound healing material in rats (Murakami et al. 2010). Like fucoidan, carrageenan also possesses the properties like biocompatibility, blood coagulation, and immunomodulator activity which help to accelerate the rate of wound healing mechanism. A pH-responsive hydrogel film based on κ-carrageenan, locust beam gum (LBG) polysaccharides, and cranberry extract (CB) powder (κC:LBG:CB hydrogel films) was constructed for wound management which showed in vitro antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Zepon et al. 2019). Nair et al. prepared ciprofloxacin loaded cyclic β-(1 → 3)(1 → 6)glucan/carrageenan hydrogels for in vivo induced wound healing purpose in rats where the porosity of hydrogel was increased due to glucan molecule enhancing the drug loading and cell attachment capacity of hydrogel (Nair et al. 2016). Zang et al. constructed pH-responsive, self-healing and injectable agarose biohydrogel demonstrating its stability and self-healing property through in vivo haemostatic test

on rabbit liver (Zhang et al. 2018a, b). The sericin encapsulated agar–sericin–glycerol-based hydrogel improved collagen production and capture free radical species to accelerate chronic wound healing (Tyeb et al. 2018).

(iii) *Tissue engineering and regenerative medicine*

In the field of tissue engineering, the polysaccharide-based polymers have extensively explored as scaffold materials. Due to the properties like hydrophilicity, ease in functionalization and strong mechanical strength, polysaccharides and their derivatives have been favored biomaterial among other which can be used as biohydrogel forming a porous network structure. Currently, the seaweed-derived polysaccharides like agarose, alginate, carrageenan, ulvan, and fucoidan are being extensively studied for tissue engineering purpose as these are biocompatible, non-toxic, biodegradable, available in excess, strong structural stability, and scope for chemical modifications. As these polymers showed promising impact in the scaffold design and cell proliferation, the various forms of polymers like fiber, hydrogel, micro or nano particles, cross-linked hydrogels, and 3D printed scaffold are prepared for tissue engineering and regenerative medicine.

The highly concentrated mixture of alginate and gellan gum was prepared to construct the 3D scaffold for the support of human mesenchymal stem cells (hMSC) which helped for proliferation and early osteogenic differentiation (Akkineni et al. 2016). Nguyen et al. designed microfluidic technique to synthesize alginate fiber where inside the alginate stream oil droplet allowed to form and gelated using $CaCl_2$ (Nguyen et al. 2020). Spheroids are generated from the culturing mesenchymal stem cells which have viability more than 95% after 14 days of culture. The tissue engineering not only comprised of the matrix supporting for tissue regeneration also carries the molecules or drugs accelerating proliferation or inhibiting infections. A fibrous scaffold consisted of a core of tetracycline loaded alginate/soy protein and shell of polycaprolactone (PCL) was prepared by coaxial electrospinning which showed promising support for the cellular regeneration along with antibacterial activity against pathogens like *Staphylococcus aureus* and *Escherichia coli* (Chuysinuan et al. 2019). The platelet-derived growth factor (PDGF-BB) required for the restoration of the vascularity in an injured region was entrapped in the polymeric beads of κ-carrageenan which showed development of fully functional vascular network in vitro (Santo et al. 2009). The encapsulation and slow release of the PDGF-BB from the beads were monitored by fluorescence microscopy revealing the potentials of this system for bone tissue engineering. The chondrocytes are encapsulated in the beads and fibers prepared from the polymeric blends of alginate and κ-carrageenan have considerable potential as cell carrier materials for cell delivery in tissue engineering/regenerative medicine applications (Popa et al. 2011). The shortage of heart donor for heart transplantation promoted the need for the cardiac tissue engineering to design a biomimetic substrate replicating the native cardiac cells. Soft lithography was used for the fabrication of agarose microplate for human embryonic stem cells (ESC) for human embryonic stem cells (ESC) aggregation where agarose acted as mold for ESC to form the

cardiac lineage facilitating stem cell-derived cardiomyocytes (Dahlmann et al. 2013). Khanarian et al. constructed scaffold composed of agarose and hydroxyapatite (HA) for calcified cartilage formation acted as a promising design strategy for osteochondral interface regeneration (Khanarian et al. 2012). Dash et al. constructed UV cross-linked ulvan-based hydrogel functionalized as mineralization inducer such as natural enzyme alkaline phosphatase to enhance calcium phosphate deposition (Dash et al. 2014). The osteogenic cells' activity was improved on the scaffolds improved binding of growth factors which are stimulating bone healing.

3D bioprinting of hydrogels with an appreciation of the native tissue architecture holds promise for generation of engineered tissues. Daly et al. constructed the 3D structure of cell laden hydrogel composed of agarose, alginate, gelatine methylacrylate (GelMA), and BioINK^{TM} and compared their varied compositions for printing properties to support proliferation of hyaline cartilage or fibrocartilage in vitro (Daly et al. 2016). Li et al. used κ-carrageenan-GelMA polymer blends for the construction of 3D structure by 3D bioprinting (Fig. 5.7) (Li et al. 2018) forming layer-by-layer assembly. The strong interaction was found due to polyelectrolyte complexes in two differently charged biohydrogel. More than 96% viability of the cells was obtained on the 3D bioprinted hydrogel. Ulvan polymer microparticles were incorporated in poly-d,l lactic acid (PDLLA) by subcritical fluid sintering with CO_2 which forming a 3D porous scaffold which showed promising support in bone tissue engineering (Alves et al. 2012a).

(b) **Remediation applications**

Naturally found seaweeds have been studied extensively for their excellent adsorption capability which was further utilized for successful removal of pollutants like dyes from the various paper, textile and printing industry, phosphorous,

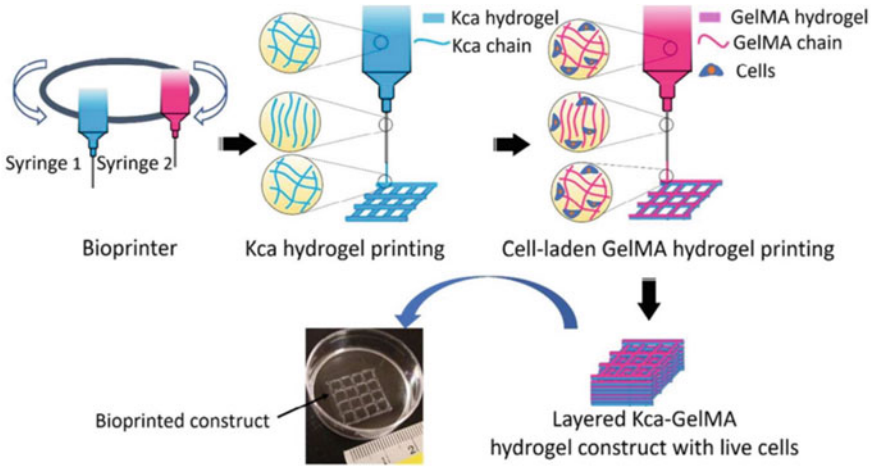

Fig. 6.7 3D bioprinting of κ-CRG-GelMA hydrogel [Reprinted with permission from Li et al. (2018). Copyright 2018 the American Chemical Society]

nitrogen, heavy metals, phenolic compounds, etc. (Arumugam et al. 2018). It is developed as alternative technology for the treatment of these pollutants due to its efficient performance and inexpensive, abundant availability of raw material. As summarized in earlier sections, the biopolymers derived from these seaweeds have potential biomedical applications. The biopolymers like alginates, agarose, agar carrageenan, fucoidans, and ulvan have been utilized directly or modified to be applied for application in wastewater treatment. Also, as these biopolymers are biocompatible and non-toxic to the biomaterials, polymers are explored for the encapsulation or stabilization for enzymes and bacterial cells.

Among all seaweed-derived biopolymers, alginate was explored extensively for direct use or with blends or with encapsulated biomaterials in wastewater treatment. Moraes et al. constructed membrane alginate–chitosan blended polymer (pristine and multi-layer model) for the adsorption of the herbicides diquat, difenzoquat, and clomazone on from contaminated water (Agostini de Moraes et al. 2013). Jung et al. used polyethylene oxide (PEO) and sodium alginate (SA) polymeric blend to remove oil from soil where the impact of soil remediation was studied by varying the polymer concentration in blend (Jung and Hu 2017). The adsorption of methyl orange and methylene blue was demonstrated using magnetic beads composed of magnetic nanoparticles and activated carbon in alginate matrix (Rocher et al. 2008). The Reactive Black 5 (RB5) dye from contaminated water was decolorized by Fe alginate gel beads through the electro-Fenton process (Iglesias et al. 2013). The graphene oxide was incorporated in agarose hydrogel to form self-assembling 3D structure for complete removal of the cationic dyes methylene blue and rhodamine B from water within 4 h (Wang et al. 2013). The silica gel was incorporated in agarose gel through dispersion to form a mixed matrix membrane (MMM) was applied for the removal and determination of the antibiotics like sulfamethoxazole (SMX), sulfamonomethoxine (SMM), and sulfadiazine (SDZ) from contaminated water sample (Rozaini et al. 2019).

The seaweed-derived polymers are the inert to the biological materials like enzymes, mammalians cells, and microorganisms. So, they provide strong matrix for the encapsulation of the pollutant degrading microbial cells or enzymes protecting the cells or enzymes from getting denatured due to direct exposure to pollutants. Alizarin red dye was discolored by chitosan–alginate microcapsule encapsulated lignolytic enzyme laccase (Lu et al. 2007). Bilal and Iqbal immobilize lignin peroxidase in the surface of Ca-alginate polymeric beads through glutaraldehyde covalent cross-linking (Bilal and Iqbal 2019). The packed bed reactor was constructed using lignin peroxidase immobilized Ca-alginate beads for decolorization of textile dye Remazol Brilliant Blue R which showed more than 80% dye removal for consecutive five cycles. Geet et al. degraded malathion in continuous packed bed bioreactor packed with immobilized *Bacillus* sp. in PVA-alginate beads (Geed et al. 2018). The scanning electron microscopy (SEM) images revealed the formation of stable biofilm during malathion in bioreactor where it was found that as flow rate increased the biodegradation rate enhanced due to the reduction of mass transfer resistance (Fig. 5.8). The white-rot fungus *Phanerochaete chrysosporium* immobilized into Ca-alginate beads for

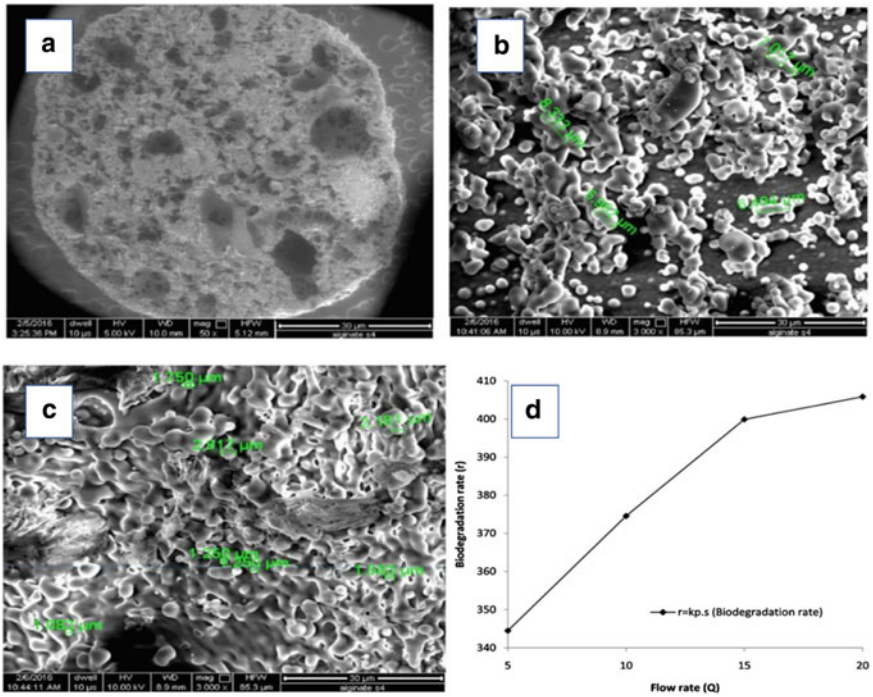

Fig. 6.8 SEM morphological images of **a** alginate beads, **b** biofilm formation before experiment, **c** biofilm formation after biodegradation of malathion in immobilized alginate beads of *Bacillus* sp. S4, and **d** effect of flow rate on biodegradation rate of malathion [Reprinted with permission from Geed et al. (2018). Copyright 2018 Elsevier]

repeated decolorization of dyes like Direct Violet 51, Reactive Black 5, Ponceau Xylidine, and Bismark Brown R (Enayatzamir et al. 2010). The palladium ion recovery from the acidic solution was also observed using *Providencia vermicola* in Ca-alginate beads (Xie et al. 2020). The low gelling temperature and weak mechanical properties are the main reasons for other seaweed polymers (carrageenan, fucoidan, ulvan, agarose) not being used for encapsulation of enzymes or microorganisms in bioremediation applications. Wang and Qian entrapped a chlorophenol-degrading microorganism in carrageenan–chitosan blended gel for repeated and efficient degradation of chlorophenol (Wang and Qian 1999).

(c) **Foodindustry applications**

The polymers obtained from the seaweeds have tremendous applications in the industries like food, pharmaceuticals, textiles, sensors, and electronics. Seaweeds are the most nutritious algal species among all form the algal species found in marine environment which is consumed in most global parts as a nutrient supplement. The extracts of seaweed have been used in many food products not only to enhance the nutritional value of the food products also to manage lifestyle-related diseases like obesity, diabetes, hypertension, etc. Currently, the polymers obtained

from seaweeds are used as additive or packaging material in food industry improving the shelf life of foods. For these applications, seaweed polysaccharide polymers like alginate, carrageenan, and agar are widely utilized due to their biocompatibility, strong gelling capacity, abundance availability, and encapsulation efficiency. These polymers are also widely used as food additives in jams, jellies, ice-creams, dairy products, etc., to improve and stabilize the structure of food. Agar has been used in Japan from the seventeenth century. Due to the high viscosity properties in the water, carrageenan is mostly used as gelling, emulsifying, thickening, and stabilizing agent in dairy products like yogurt, ice-creams, cheese, etc. Roohinejad et al. reviewed in details the use of polysaccharide polymers from seaweed in food products (Roohinejad et al. 2017). The seaweed-based composite films and coatings are also used for food packaging alternating to the existing plastic films. Huq et al. produced alginate-based film reinforced with nanocrystalline cellulose for food packaging material which showed increased tensile strength and decreased water vapor permeability (Huq et al. 2012). Martins et al. constructed polymeric film composed of based on κ-carrageenan/locust bean gum having antimicrobial activity against *Listeria monocytogenes, Escherichia coli and Salmonella enterica* which was used for enhancing the shelf life of the food (Martins et al. 2013). Agar–nanocrystalline cellulose–savory essential oil blended film showed improved mechanical, water absorbing capacity which has antimicrobial activity against gram positive and gram negative microorganisms improving the shelf life of the food products (Atef et al. 2015).

6.5 Conclusion

The biopolymers have shown their potential advantages over petroleum-based polymers due to their renewability and biodegradability. These environmental-friendly polymers influenced consumers to adapt lifestyle to use these biopolymers in their daily usage. The advancement in the biosynthesis and fermentation process increased possibility to increase the production of biopolymers in sufficient quantities to use them commercially. The seaweeds are also now large source of biopolymers as these seaweeds can be cultivated very easily, in all environmental conditions, producing abundance form of biopolymer. These seaweed-based polymers have been extensively studied to optimize the procedure for polymer extraction, formation of film or gels, construction of the blends with other natural polymers and applied these polymeric materials in various industries like food, textile, pharmaceuticals, clinical, remediation, health care, etc. The extensive research for more than three decades has come to inference that the seaweed-derived biopolymers and their composite/blends are highly stable, biocompatible, and non-toxic to the biological materials in vivo and in vitro. Despite the government legislation on the use of petroleum-based polymers like plastic and consumers demands for renewable materials, the industrial production of the biopolymers is very limited due to their overall cost of the large productions

compared to traditional plastic like polymers. The improvement and optimization of the downstream and upstream strategies need to minimize the cost of production and recovery of biopolymers. The bacterial PHA production is another biopolymer industry which is immensely contributing and modifying the commercialization of the biopolymers. The few seaweeds are also major source of PHA polymers which can be cultivated in freshwaters. The role of biotechnologist is very crucial in mass production of PHA using genetic transformation. The metabolic pathway of recombinant *E. coli* is modified for the large accumulation of PHB which is an ideal example to amplify the seaweed biopolymer production in microbial species.

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Chapter 7
Seaweed Cultivation and Its Biobusiness Status Around the World

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7.1 Introduction

Seaweeds or macroalgae, a highly useful and simple type of plants, lack true roots, stems and leaves. Heavy loads on numerous usual resources impose the development of substitute sources to produce significant goods such as food, food additives, feed, fuel, maquillages, and antibiotics. The improvement of large-scale seaweed aquaculture has the prospective to play a significant role in meeting future resource needs. The seaweed is an important character of culture and society (Delaney et al. 2016). Seaweeds, the simplest and primitive plants, largely scattered over the sea from tidal level to substantial depths, floating freely or sometimes devoted to substrate with a holdfast. These are wonderful plants of the sea as they efficiently converted solar energy and grow faster than anything on earth (Kumar et al. 2016). Seaweeds involve macroscopic, multicellular and marine algae species of class *Rhodophyta* (red), *Phaeophyta* (brown) and *Chlorophyta* (green) macroalgae (Odom et al. 2000). The marine organisms (microalga) are the potential producer of certain bioactive compounds, secondary metabolites and energy sources.

About 1700 years ago, the earliest written record of the interaction of macroalgae for human usage originated from China (Yang et al. 2017). Initially, these marine algae were used for certain domestic purposes (food and feed), but later on, these found their importance in industries (Delaney et al. 2016; Ganesan et al. 2019; Johnson et al. 2017). In the sixteenth century, Chinese herbal producer used brown algae for treatment of goiter, *Gelidium* for duodenal illnesses and *Laminaria stipes*is used during childbirth for the dilation of the cervix (Levine

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2016). Some reports demonstrated that Japanese use seaweeds as a part of their diet 300 BC. Southeast Asian countries, i.e., Japan, Korea, China and presently USA and Europe are main seaweed utilizers. The Republic of Korea emerged as the highest consumer of seaweed utilizer worldwide for minerals and vitamins (Kumar et al. 2016).

In Europe, people started cultivating and collecting sea algae in 100 BC. They use this marine food to feed animals, dyeing fabrics and also as medicine for some ailments. Microalgae have been collected for food and fertilizer in Ireland in 1200 AD. The first book written about seaweeds, and their uses were written by a Chinese author (Rose 2016). Aquaculture is a fresh and healthy form of marine culture. World fabrication in the field of seaweed production is growing exponentially from the last 50 years (Loureiro et al. 2015), and it increased rapidly, i.e., from 7 to 24 million tons between 1997 and 2012 (FAO 2016a). The substantial increase in demand and production for seaweed cultivation is an indicator of their prospective as an important match to crops. Sea can also be converted into eco-friendly sophisticated fields using innovative technologies for massive seaweed biomass production and its multiple uses, besides coastal areas. Seaweeds for variety of uses can be cultivated without landfields, freshwater or fertilizer. Additionally, it will also provide a variety of ecosystem amenities having ecological and economic importance (Chopin 2014; Radulovich et al. 2015).

Diverse and multipurpose biomass is produced by seaweed having multiple applications. These can be used in many forms, i.e., fresh, dried, powdered, canned and packed solely for human consumption. Also used as food additives, feeds, fertilizers, cosmetics, biofuels, medicines and nutraceuticals (Sudhakar et al. 2019; Anis et al. 2017). Products produced from seaweed, i.e., agar-agar, alginate and carrageenan play an abundant role in many leading industries like food, beverages, chemical, pharmaceutical, cosmetics and textile industries. Most important and valuable commercial role of seaweed after used as food is that it provides the raw material for extraction of hydrocolloids and phycocolloids (Veeragurunathan et al. 2019). Global demand for seaweeds as potent biomass for a variety of new uses beyond traditional applications has been increasing tremendously (Rebours et al. 2014; Hafting et al. 2015). Most commonly cultivated seaweed such as *Eucheuma* sp. *Kappaphycus alvarezii*, *Gracilaria* sp., *Saccharina japonica*, *Undaria pinnatifida, Pyropia* sp. and *Sargassum fusiforme* used for production of carrageenans and agar. Harvesting is the most important step to obtain microalgal biomass for the manufacture of targeted products. Low-cost harvesting is one of the key bottlenecks in seaweed production sector as it is the difficult, costly and energy-consuming process (Mac Monagail et al. 2017). So, industrial utilization of seaweed needs to find an effective, appropriate and inexpensive method for the harvesting of biomass. Seaweeds can be produced industrially with certain different technologies but at the ground level optimization and efficient development for higher production are still in its infancy phage. This chapter is emphasizing on the prodigious possibilities of seaweeds biomass as raw material for industrial-scale production for various value-added products.

7.2 Global Scenario of Seaweed Production

The production of the seaweed is good for aquaculture industry, economy as well as for the ocean as it is environmentally friendly and does not need extra feed and attention; moreover, it also improves water quality. Production and harvesting of seaweed also open new opportunities and income avenues to the coastal population. In the year 2005, total 14.7 million tons of seaweed was produced worldwide; of these, 13.5 million tons was cultivated and 1.2 million tons was harvested from wild types. After ten years, the production of seaweeds doubled, i.e., 30.4 million tons at the end of 2015 (29.4 million tons cultivated and rest harvested from wild algae) (Ferdouse et al. 2018). The commercial market for seaweeds is predicted to grow at a fast rate, i.e., CAGR of 8.4% from 2018 to 2023, to range up to 21.1 billion USD by the end of 2022 (G.A.A. 2017; CSM 2018).

The largest seaweed producers are China (50.1% of total production), Indonesia (35.6%) and Philippines (5.8%) with more than 23 million tons of production in 2014. (Buschmann et al. 2017). Southeast Asia and non-Asian European also consume seaweed as food. The USA has considered seaweed as an additional source of nutritious food and considered it as "food for health." USA market has strong consumer inclination toward organic, sustainable seaweed products. These products have low or very fewer influences on both the environment and biodiversity (Chapman et al. 2015). Besides this only 1% of total seaweed biomass is used globally for food and other uses (additional to hydrocolloids). In Europe, *Undaria* is cultivated primarily and has developed as an industry under the mariculture. European Union's Blue Growth initiative policies provide subsidies to push the study the possibilities in the seaweeds industry, specifically their usage in biotechnology (Delaney et al. 2016; Venkatesan et al. 2017). The Indian history of seaweed cultivation and research is almost forty years old and was last reviewed in 1998. After that a lot of new statistics related to resources, utilization and commercial cultivation of seaweed has been added (Rao and Mantri 2006; Mantri et al. 2020). The *K. alvarezii* (Philippines origin) is the first commercially cultivated Indian seaweed procured form Japan (1984) by CSIR-CSMCRI. During 1995–1997, *K. alvarezii* cultivation started in India after 9 years of experiments by CSIR-CSMCRI at Mandapam in the southeast coast of India. Its commercial cultivation started in 2003 at Tamil Nadu. At present, it is cultivated in five coastal districts (Ramanathapuram, Pudukottai, Thoothukudi, Thanjavur and Kanyakumari) of Tamil Nadu (Abhilash et al. 2019). Mostly tropical species of seaweed are cultivated in India, but some cold, moderate, and subtropical rudiments have also been reported. A total of 20,000 types of marine algae have been recorded in India (Tandel et al. 2016), and of these about 105 species were cultivated in Andaman and Nicobar only. The most common species of sea algae are *Acetabularia calyculus, Codium taitense*, *Halimeda* sp., *Dictyosphaeria cavernosa, Padina pavonica, Pocockiella* sp., *Turbinaria* sp., *Galaxaura* sp. (Mohanraju and Tanushree 2012). The recent studies from the Indian region approximately documented 865 seaweed taxa, a very few of these taxa are well characterized, and there

is need to examine many of them for microscopic and molecular characterization (Mantri et al. 2020). A total of 302 species of seaweed were cultivated in Tamil Nadu 202 in Gujarat, 152 in Maharashtra, 89 in Lakshadweep, 78 in Andhra Pradesh, 75 in Goa, 39 in Karnataka, 34 in Andaman & Nicobar Islands, 20 in Kerala, 6 in West Bengal and 1 in Orissa and 34 in Andaman and Nicobar (Tandel et al. 2016) (Table 7.1). About 22,000 tons of macroalgae were annually produced in India. Commercial cultivation of macroalgae in India has just begun and facing certain monitoring hurdles. Processing of macroalgae is limited to produce low grades of agar-agar and alginate.

7.3 Resources of Seaweeds

Many R&D programs have been launched to discuss the possibility of using marine biomass as a source of energy (Bird and Benson 1987). Seaweeds are an integral part of coastal ecosystems and offer valuable services to support the life of many living forms (Levine 2016). Seaweeds are used for food, phycocolloid production, fertilizers and extraction of biologically active compounds, that stimulates the growth of microbes, plants, animals and humans. With an increase in the cultivation of seaweeds, its resources also increase. The taxonomically diverse seaweed is biogeographically interesting, ecologically multifarious, socially significant and cost-effective (Haws et al. 2019). The resources of seaweed are distributed based on places and their characteristics.

7.3.1 Geographically Distribution of Seaweed

a. **Seaweed resources of China**: China is largest producer of seaweeds. The aquaculture started in 1952 in China and increased steadily. *Laminaria japonica* is most widely cultivated and economically important seaweed in China. *Laminaria*, *Gracilaria* sp., *Sargassum fusiforme*, *Eucheuma muricatum*, *Prophyra* and *UlvaLactuca* are seven mainly cultivated seaweed sp. of China (Zhang 2018). All these species were cultivated, harvested sun dried and directly consumed as food. Beside these *Gloopeltis*, *Dunaliella*, *Spirulina* and *Undaria* were also cultivated in China by traditional stone-throwing method (Tseng 2001).
b. **Seaweed resources in Indonesia**: Indonesia is second largest seaweed producer after China; it is situated between Asian and Australian continents, between the Pacific and the Indian Oceans. *Eucheuma* is most widely cultivated seaweed sp. with annual production of tons (White and Wilson 2015). Besides this *Gracilaria lichenoides*, *Hypnea* sp., *Ulva* sp., *Laminaria* sp., *Sargassum* sp., *and Carragreenophytes* were also cultivated in Indonesia (Soegiarto 1990).
c. **Seaweed resources of Israel**: The marine diversity of Israel in the Eastern Mediterranean Sea (EMEDS) is significant; they cultivated more than 300

Table 7.1 Commercially cultivated seaweeds species in India

S. no	Name of marine algae	Use	Method of cultivation	Place of cultivation	References
1	*Ulva flexuosa*	Antibacterial activity	In plastic pools	Okha	Mohanraju and Tanushree (2012)
2	*Ulva lactuca*	Food, feed and antiviral activity	Seeding of spore Suspension on nets	Okha, Kerala	
3	*Hydropuntia edulis*	Food grade agar	Long-line rope method	Diu, Ervadi, Southeast coast, Krusadai Island	Tandel et al. (2016)
4	*Gelidiellaa crosa*	Bacteriological agar	Coral Stone	Ervadi	Sharma et al. (2017)
5	*Hypnea musciformis*	Carrageenan	Single raft floating technique	Ervadi	
6	*Gelidiella corticata*	Anticancer, anticholinesterase, antifertility and anticoagulation	Raft technique	Saurashtra, Kerala	
7	*Gelidiella textorii*	antimicrobial and antioxidant	Raft culture method	Andhra Pradesh	
8	*Gracilaria compressa*	Agar, antibacterial	Raft floating technique	Tamil Nadu	
9	*Gracilaria debilis*	Agar-agar	Raft culture method	Tamil Nadu	
10	*Gracilaria edulis*	Animal feed, fertilizer and wastewater purification plants	Floating bamboo raft, net bag, hanging rope technique	Narakkal, Kochi	
11	*Caulerpa acemosa* (Forssk)	Food additive, pharmaceutical and cosmetic products	Net bag	Gujarat	

species of total existing (1200) species (Lipkin and Friedlander 1998; Israel and Einav 2017; Badreddine et al. 2018). In spring and winter, the algal communities on the seashore are abundant with high-standing frameworks emerging on abrasion platform (Fig. 7.1) (Einav and Israel 2007). Israel has significantly contributed to the R&D of aquaculture and currently two companies processing the number of products with *Gracilaria* and *Ulva* and sold it locally and internationally.

d. **Seaweed resources of Philippines**: In the Philippines, more than 600 algal species reported from which two carrageenophytes, i.e., *Kappaphycus alvarezii* and *Eucheuma denticulatum* are majorly cultured (Silva et al. 1987). Besides

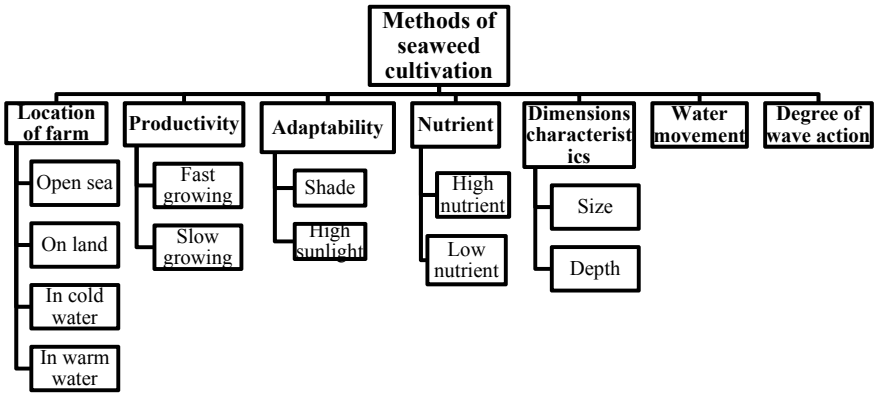

Fig. 7.1 General layout of different seaweed cultivation methods

these two, *Caulerpa* is also utilized and cultivated for food. These seaweeds and its products are exported to different countries. In 2016, US$198 million were earned from seaweed. *Caulerpa lentillifera* is one of the preferred (due to its succulent texture) and only green seaweed food additive commercially cultivated in the Philippines (White and Wilson 2015).

e. **Seaweed resources of USA**: Alaska, the largest coastlines in USA, has more than 500 species of marine flora. There are species of floating kelps occur from the southern frontier to Kodiak Island and westward beside the Aleutian chain, *Fucus* sp, are the most dominant microalgae in the main intertidal coastline. There are special resources of Alaskan native subsistence, i.e., "Black seaweed" (*Pyropia* sp., *Saccharina latissima* and *Alaria marginata*). Alaska has lots of seaweeds which are good resources for the state and nation if well settled (Stekoll 2019).

f. **Seaweed resources of Hawaiian Islands**: More than 522 seaweed species were cultivated in Hawaiian Island; among these, 345 belongs to phylum Rhodophyta, 107 to Chlorophyta and 62 to Ochrophyta (Abbott 1999). In tropical Indo-West Pacific phytogeographic region, boreal species (*Desmarestialigulata*), endemic species (*Codium Hawaiians*) and species with disjunct distributions were cultivated (e.g., *Nereiaintricata* Yamada) (Abbott and Huisman 2003; McDermid and Abbott 2006). For everyday life in The Hawaiian Islands, *limu* (Hawaiian word for a plant growing in a wet place) was used as a regular meal. There were more than 63 species of *limu* cultivated and utilized by Hawaiians for their domestic use (Abbott 1996) (Table 7.2). Nutritional composition of *limu* is more than the three main food items: fish, taro and breadfruit. Hawaiian seaweeds also known to have measurable amounts of nutritional elements, i.e., vitamin, protein, fats and dietary fiber (McDermid and Stuercke 2003, McDermid et al. 2005).

Table 7.2 Commonly utilized seaweed species in Hawaiian

Phylum	Species	Hawaii name	Uses	References
Rhodophyta	*Asparagopsis taxiformis*	*kohu*or *līpehe*	Human food and animal fodder	Abbott (1999)
	Ahnfeltiopsis concinna	*ʻakiʻaki*	Remove fine lines, wrinkles, enlarged pores and loss of firmness.	Abbott and Huisman (2004)
	Gracilaria coronopifolia	*Manauea*	Human food and effective against viral infections	
	Gracilaria parvispora	*Ogo*	Human food	McDermid et al. (2005)
	Halymenia hawaiiana	*lepe-o-Hina*	Human foods, drugs, fodder, used as a natural coloring in cosmetics, pharmaceuticals and food	
	Laurencia nidifica	*Māneoneo*	Condiment due to its taste, insecticidal	McDermid and Abbott (2006)
	Laurencia dotyi	*līpeʻepeʻe*	Food species and eaten with raw fish	
	Laurencia succisa	*līpeʻepeʻe*	Human food and eaten with raw fish	
	Pyropia vietnamensis	*paheʻe*	Halt the metabolism, increase lipid production to fight against diseases	Shi et al. (2015)
Chlorophyta	*Codium edule*	*wāwaeʻiole*	Salad, animal fodder and insect repellent	
	Codium reediae	*aʻulaʻula*	Stepal food eaten with fish stew, or salad	Ishii et al. (2017)
	Ulva lactuca	*Pālahalaha*	Soups, salads, adornment in traditional hula	Kavale et al. (2018)
	Ulva prolifera	*ʻeleʻele*	Food, functional ingredients	

g. **Seaweed resources of India**: Seaweeds are mainly used and cultivated for the agar and alginate production in India. *Gelidiellaacerosa* (Forsskal) and *Gracilaria edulis* are harvested for agar production, while *Sargassum* sp. and *Turbinaria* sp. are the source material for alginate production. Due to high carbohydrates content, the green algae *Ulva* sp. and other seaweeds have phycocolloid or cellulose content which makes these best bioresource for biofuel production (Trivedi et al. 2016).

7.3.2 The Characteristic Distribution of Seaweed

a. **Chlorophyta**: Chlorophytes are also known as green algae, most common habitat in freshwater, some species also found in the ocean. Thousands of unicellular and multicellular species of macroalgae with branched or unbranched filaments were reported. The cellulose and chloroplasts are the main component of cell wall of members of these phyla. The chloroplast contains Chlorophyll a, Chlorophyll b and β-carotene for photosynthesis along with starch and oils; cell wall of green algae is made of as main storage product. They reproduce by non-motile aplanospores and zoospores having one flagellum (Kavale et al. 2018) (Table 7.3).
b. **Xanthophyta**: Most commonly known as yellow-green algae, most commonly found in freshwater, but also inhabitant in saltwater and wet soil environment. These 450–650 species are the least dynamic species of algae. The cellulose is main component of cell wall of these unicellular organisms along with silica. The chlorophyll a, chlorophyll c, β carotene and xanthophylls are the pigments presents in the choloroplasts of xanthophyta (Romera et al. 2007). The motile zoospores or non-motile resting aplanospores are the main reproductive spores.
c. **Bacillariophyta**: Members of this family are commonly known as diatoms. These are the most abundant types of unicellular algae, found in both fresh as well as saltwater. These having pectic and silica as main cell wall substances, silica formation called a frustule. Chlorophyll a, chlorophyll c, β-carotene and fucoxanthin are chlorophyll and carotenoids present with chrysolaminarin and oils are storage product of diatoms. Diatoms reproduce approximately after every 24 h by asexual multiple fission (Franklin 2004).
d. **Phaeophyta**: This is the largest group of marine multicellular algae also known as brown algae. These having kelp of the order *Laminariales*, 30–60 m (200 ft) in length and forms conspicuous underwater kelp forests (Cock et al. 2011). Brown algae contain the pigment fucoxanthin, and its cell wall has cellulose and alginic acid. They mainly reproduce by flagellum, spore and gamete. Brown algae include the fastest growing seaweeds (Connor and Baxter 1989).
e. **Rhodophyta**: Also known as red algae due to the presence of zeaxanthin with phycoerythrin and phycocyanin as phycobiliprotein which makes them appear red. These are commonly found in tropical marine locations and need solid surfaces for growth, including tropical reefs or sometimes attached to other algae. The cellulose, carbohydrates, chlorophyll a and Chl d are the main constituents of cell wall. Thenon-flagellated monospores are asexual spores, and sexual reproduction takes place by mean of under alternation of generation. Large number of seaweed belongs to this phylum (Romera et al. 2007).
f. **Chrysophyta**: Also known as golden brown algae due presence of fucoxanthin. These algae mostly found in freshwater and having two flagellates. They reproduce by motile which divide by fission, while non-motile forms produce motile zoospores.
g. **Euglenophyta**: Most commonly called as euglenoids especially found in fresh as well as in marine water. These are unicellular and flagellated without a cell

Table 7.3 Cellular characteristics of major groups of algae

Algal group	Body form	Cell wall	Chlorophylls carotenoids	Storage products	References
Green algae	Unicellular or multicellular, branched unbranched filaments	Cellulose	Chl a, Chl b and β carotene	Starch and oils	Douglas (2002)
Yellow-green Algae	Unicellular or coenocytic filaments	Pectic substances	Chl a Chl c β carotene and xanthophylls	Chrysolaminarin, oils	Franklin (2004)
Diatoms	Unicellular	Pectic substances and silica	Chl a Chl c and β carotene fucoxanthin	Chrysolaminarin, oils	
Brown algae	Multicellular	Cellulose and alginic acid	Chl a Chl c,β carotene and fucoxanthin	Laminarin, oils	Romera et al. (2007)
Red algae	Multicellular	Cellulose	Chl a Chl d, β carotene and zeaxanthin	Floridian starch	
Golden brown Algae	Unicellular		Chl a Chl c, fucoxanthin, lutin	Chrysolaminarin,	Cock et al. (2011)
Euglenoid flagellates	Unicellular		Chl a and Chl b	Paramylum oils	
Cryptomonads	Unicellular	Cellulose (cell wall present)	Chl a Chl b and alloxanthin	Starch and oils	Kavale et al. (2018)
Dinoflagellates	Unicellular, motile (flagella)	Cellulose plates covering the cell	Chl a Chl c dinoxanthin	Starch and oils	

wall. Characteristics feature of photosynthetic euglenoids includes an eyespot, flagella and organelles (nucleus, chloroplasts and vacuole) include them in phylum *Euglenophyta*. Paramylum and oils are their storage products.

h. **Cryptophyta**: Also known as cryptomonads and found in freshwater but some species also present in brackish water (Kent and Barnes 2001). These are unicellular and found with or without a cell wall. They have alloxanthin pigmentation, and phycobiliproteins are phycoerythrin and phycocyanin (Douglas 2002).
i. **Pyrrophyta**: Dinoflagellates are also known as fire algae, mostly found in oceans and some of freshwater, and form phenomenon of red tides as the oceans appear red and also of bioluminescence. These are unicellular and cell wall covered with cellulose plates. They have chlorophyll a and c with dinoxanthin pigmentation.

7.4 Nutritional Composition

The nutritional content of these edible seaweeds varies from species to species like fiber, mineral, fatty acids, vitamin and protein with different concentrations (Table 7.4). But these contents are not fixed; it may be vary according to season and sampling techniques (Ito and Hori 1989).

7.4.1 Fiber

Fibers cannot be easily digested in the gut. The digestion of seaweed fiber occurs by the fermentative capacity of the lower intestine and their passage through the gastrointestinal tract (Brownlee et al. 2005). Fibers are of two types as insoluble and soluble, the daily requirement of dietary fiber is 24 g per day, 8 g of seaweeds contains 12.5% fibers sufficient to fulfill the requirement of a person. Seaweed becomes a large component to provide proper diet fiber as compare to other vegetables (Brownlee et al. 2005).

7.4.2 Mineral

Due to marine habitat seaweeds have lots of minerals, calcium is present in highest concentration in comparison to terrestrial stuff (Ana et al. 2018). Along with higher calcium concentration, the concentration of iron and copper is also higher than in various well-known terrestrial food sources such as meats and spinach (McCance et al. 1993). Brown seaweeds are a good source of magnesium, copper, iron and iodine as well as other rarer elements. For metabolic regulation and growth patterns, iodine is best to consume and is abundant in most seaweeds (Garrow et al. 1997). Other trace elements like zinc and arsenic also present in seaweed but very low concentration.

7.4.3 Fatty Acids

About 2% of total dry weight of seaweeds consists of lipids which are made up of polyunsaturated fatty acids (PUFA). The omega-3 and omega-6 fatty acids were observed in most of the microalgae; these were found to be essential fatty acids for humans (Sanchez et al. 2004). Besides these other essential fatty acids were also observed in seaweeds which are essential dietary component of human diet (Dembitsky et al. 2003).

Table 7.4 Biochemical content of some commonly used seaweeds

Seaweed		*Ascophyllum nodosum*	*Laminaria digitata*	*Undaria pinnatifida*	*Porphyraum bilicalis*	*Palmaria* sp.	References
Fiber (mg/8 g) and minerals (mg/8 g)	Total fiber	2.80	3.00	2.80	2.70	2.70	Brownlee et al. (2005) and Ana et al. (2018)
	Ca^{2+}	184.00	176.80	93.60	24.00	74.40	
	K^{+}	244.80	976.00	52.00	212.00	584.80	
	Mg^{2+}	72.00	195.60	65.60	76.00	48.40	
	Mg^{2+}	375.60	32.80	374.00	84.00	172.60	
	Cu^{2+}	0.30	0.14	0.16	0.07	0.20	
	Fe^{3+}	4.70	22.10	3.26	42.00	6.40	
	I^{-}	5.80	34.00	3.20	670.00	1.30	
Vitamins (µg/8 g)	A	3.00	782.00	988.00	544.00	638.00	McCance et al. (1993) and Norziah and Ching (2000)
	B1	69.00	5.00	336.00	54.00	12.00	
	B2	19.00	5.00	780.00	192.00	40.00	
	C	209.20	1378.00	192.00	9040.00	2720.00	
	E	29.00	275.00	1392.00	114.00	1296.00	
fatty acids (µg/8 g)	Saturated	39.06	33.82	20.39	60.48	64.95	Sanchez (2004)
	Unsaturated	22.75	19.23	10.50	10.67	28.86	
	PUFAs	38.16	46.94	69.11	28.86	16.11	
Bioactive components (g/g)	Alginic acid	0.28	0.32	–	–	–	Holdt and Kraan (2011)
	Fucoidan	0.11	0.55	–	–	–	
	Laminarin	0.45	0.14	–	–	–	
	Mannitol	0.75	0.13	–	–	–	

7.4.4 Vitamins

Most of the seaweeds present in water, but most of its part remains exposed to sunlight, due to which it possess antioxidants and vitamins (McCance et al. 1993). Both water-soluble and fat-soluble vitamins are present in seaweeds. Fat-soluble vitamins A, B, C, and E are present, as in Vitamin E, an important antioxidant. Concentration of water-soluble vitamin (vitamin C) is higher in *Ulva* sp., *Undaria pinnatifida*, and *Gracilar*ia sp. (Fan et al. 1993), and these seaweeds are also become one of the few vegetable sources of vitamin B12.

7.4.5 Protein and Bioactives

Seaweed consists of about 47% of protein of its total dry weight, although its concentration may varies according to season and species (Fleurence 1999). Aspartic and glutamic acids amino acids in the proteins enhance the flavor and taste of "Umami." Monosodium glutamate is most abundantly found in *L. japonica*; this was found its use in cooking in Asian continent (Marcus 2005). *Palmaria palmata* (Dillisk/Dulse) and *Ulva* sp. also contain various essential amino acids (histidine, leucine, isoleucine and valine). The biliproteins and fluorescent markers (phycoerythrin) are the major light-harvesting pigments in rhodophytes and used in scientific experiments (Wozniak et al. 2016). The other biliproteins are phycocyanin and allocyanin used for absorbing light in different wavelengths, i.e., 450–650 nm. Other than this some bioactive compounds also present in seaweeds like alginic acid, mannitol, fucoidan, floridoside, laminarin, porphyran and pentoses.

7.5 Production of Seaweed

Globally, 18% of the total protein, i.e., 56 million metric tons, is produced by the seaweed. But the seaweed abundance, quality and distribution are depleting due to climate change (Straub et al. 2016). Thus, the seaweed farming is the best way to revive this resource. Seaweed farming is an alternative form to improve economic conditions and to avoid the fisheries overexploitation (Arbystrom et al. 2007). Seaweed farming is the least environmentally damaging act in aquaculture as these practices do not require additional fertilizers and other chemicals. The seaweeds which mainly occur in the marine coastline are cultivated for biomass production (Tseng 1981). The nori, kelps, carrageenophytes and macrophytes are well-known seaweed cultivation industries along with the partnership to basic science and user demand for products. To increase the production of seaweed, the marine techniques should be improved and will need the upgradation of demand for commercial products in the market and improves the pre and post-harvesting (Cordero et al. 1993).

During past 70 years, the seaweed farming increasing rapidly, most commonly in Asia and most recently in the Americas and Europe. It is quite challenging to be acceptable by the science and society, because of certain cost-effective, thermo-tolerance, disease resistance, and fast-growing strains that can tolerate in the stressed environment conditions. The total world annual production of aquaculture increased by 20% during past few years (Bjerregaard et al. 2016; Cottier-Cook et al. 2016); most of the fabrication arises in Asia. Seaweed farming is increasing by more than 81% of total production with the use of the brown kelps (*Saccharina japonica* and *Undaria pinnatifi*), and the red seaweeds (Kim et al. 2017). All the seaweeds are produced from seaweed farming as it is a practice of cultivating and harvesting of seaweed (Borgese 1980).

7.6 Methods of Seaweed Farming

There are lots of methods of seaweed cultivation as it varies to each species according to the conditions shown in Fig. 7.1. According to the condition, cultivation methods change with the cost-effectiveness and the application of seaweed in the ecosystem. With all conditions, the cultivation methods are divided into extensive and intensive cultivation.

7.6.1 Extensive Cultivation

In order to get large crop of seaweeds with definite properties like polysaccharides, smell and taste extensive cultivation required in natural area with sunlight, water, heat and motion and can be used in algo therapy, medicines or dietary supplements (Babenko 1981). In the 1990s, various strategies and method had been developed for the cultivation of agar-producing seaweed *Ahnfeltiato buchiensis* (Titlaynov et al. 1995). It grows very slowly, live within a wide range of light intensity, from 80 to 0.1% incident photosynthetically active radiation are present. Some measures are taken for the production of algae:

1. Every the year the characteristics of an algal bed should be assessed and also the configuration of the field and the areas of production and harvest plots. The annual net production and allowable harvests should be calculated for the whole bed and each harvest plot.
2. Production plots and harvest plots are present separately. A production plot is a basic (reaching 70–95% of the total area) part of a field where algae regularly torn from it by storm waves and carried away or washed ashore. On the other hand, harvest plot is an area where algae accumulate, brought by wave action.

3. Annually, the seaweed harvesting is calculated with the several past year's data taken in account (distribution, biomass, and primary production of the seaweeds). Every year, about 10% of the total biomass of an *Ahnfeltia* bed checked so that there was no decline in seaweed resources for several years.
4. At ashore or float near the shore algae should be collected. The detachment of pieces and washing ashore of an *Ahnfeltia* bed is a natural process for a mature highly productive algal field. The natural removal of these pieces, the algal bed refreshes, its thickness is reduced, and the yield increases. Within 2 or 3 days, the washed biomass of *Ahnfeltia* should be collected and processed.
5. Collection of seaweed without destroying the field or even changing its size. The field will roll up by its self because of having no breaks. Natural seaweed fields should be expanded, and new fields formed and the expansion of seaweed fields is carried out by transporting tufts of *Ahnfeltia* to the bottoms of straits and small bays and then they buy cultivated with the help of some techniques or naturally (Titlyanov and Titlyanova 2010).

7.6.2 Large-Scale Commercial Mariculture

The commercial cultivation is done by naturally or artificial use of substrate for the production of seaweed bed. The commercial cultivation is done by seabed (*Gracilaria* spp.), on lines and ropes (*Kappaphycus* spp., *Eucheuma* spp., *Hypnea musciformis*) and nets (*Porphyra* spp., *Gracilaria* spp., *Cladosiphon okamuranus*, *Ulva* spp.) (Buschmann et al. 2001).

a. **Seaweed cultivation by seabed method**: Seaweeds are grown in the seabed by cultivating the pieces of thalli anchored to the bottom in shallow lagoons and bays, and it should be protected from winds as shown in Fig. 7.2 (Santelices and Ugarte 1990). After several months, seaweeds were harvested before the formation of reproductive organs.
   Instead of this, the plantlets directly buried in the bottom or tied to pieces of corals (Alveal 1986), and sometimes the seaweed were grown on the seabed without fixing them to the substratum, but attached with fences, so it will not be disturbed by the wind or shore (Glenn and Doty 1990).
b. **Seaweed cultivation by ropes or lines**: Seaweed farms may be located in the open area and closed lagoons, were on lines or ropes suspended at the surface of the water or several meters below the surface. Now on ropes or lines made of polypropylene, the seaweed is tied and then the ropes are tied to buoys or rafts and are anchored to the bottom (Titlaynov et al. 1995) shown in Fig. 7.3. Seaweeds are arranged in parallel rows on ropes with several to hundreds of meters at intervals from 10 cm to 1 m. After sometimes the seaweeds are harvested from the ropes to use it for different products.
c. **Seaweed cultivation on the net**: The net's mesh size and its thickness depend on the external morphology of plants. In the intertidal or subtidal zones, the nets

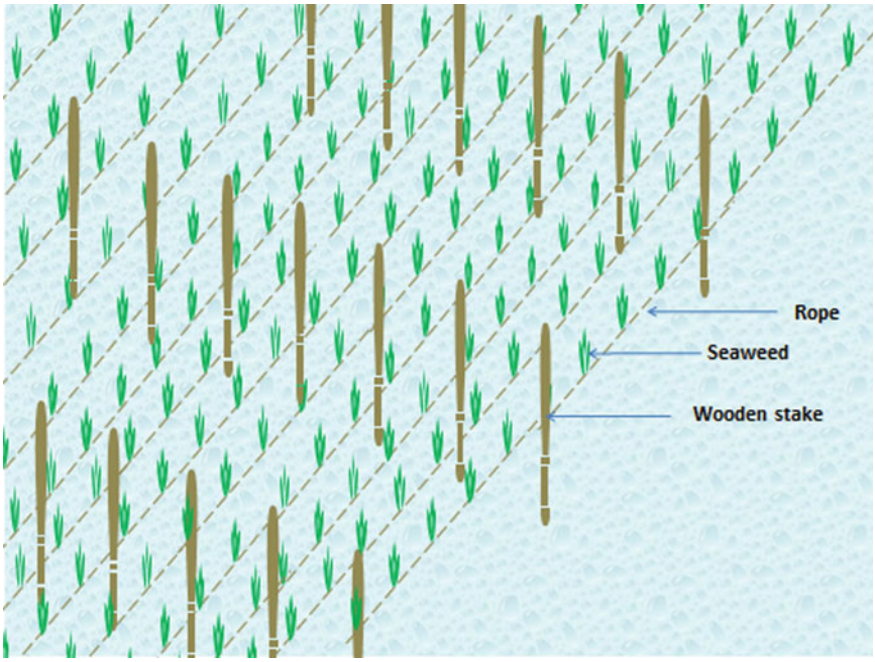

Fig. 7.2 Seaweed cultivation technique by ropes or lines

were disposed and attached with bamboo and then bamboo stacks put into the sea bottom suspended by floats shown in Fig. 7.3. In the cultivation cycle of *Ulva* and *Monostroma,* the seaweed collection occurs from nets were set up over algal beds in the period of active sporulation and special tanks the indoor spore collection in the water with spores obtained from microsporophylls reared in nurseries (Critchley 1993).

7.6.3 Intensive Cultivation

Intensive cultivation means a lot of money, and labor is required to increase the seaweed yield per area of land. There are different types of methods in which first is to cultivate the seaweed species in tanks using natural or artificial sunlight, phytohormones and nutrients, and other one is to cultivate in small water bodies like ponds, lake, etc. using organic or inorganic fertilizers with light and water motion (Neori et al. 1991).

a. **Seaweed cultivation in tanks**: Tank cultivation is the best cultivation methods among all, due to its highest yields (per 1 m^2 of water surface) cultivation. To purify the effluents from cultivated animals, seaweeds are cultivated in tanks and

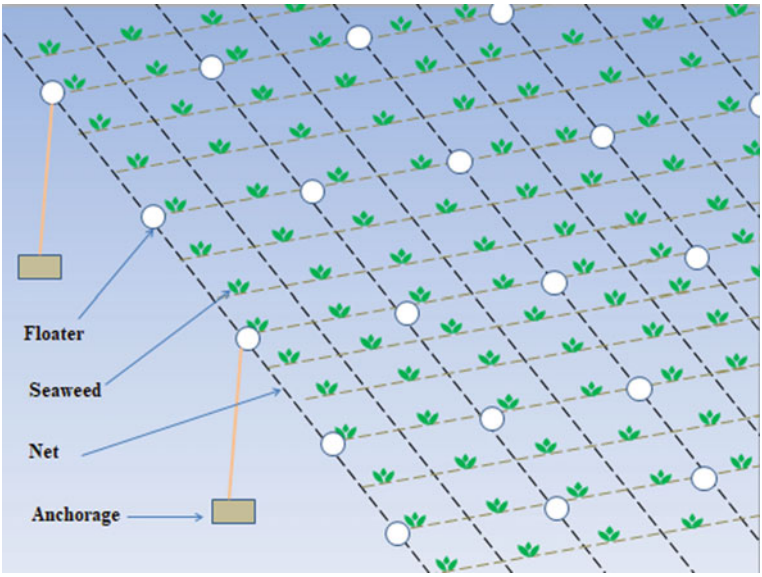

Fig. 7.3 Seaweed cultivation technique by floating net method

grow plantlets for further cultivation to get valuable products (Huang and Rorrer 2003). In-tank cultivation methods, lots of expensive materials and equipment are required; it is costly but the yield is high in this method. It required light filters, screens and additional lamps for the proper light intensity; it also required optimal irradiation, temperature and nutrition conditions. The rotation of seaweeds in tank is done by the cyclic rotation from the bottom to the water surface by air injected into the tanks. Temperature is maintained by cooling or heating in the tanks. Fertilizers are added constantly or at regular intervals with salinity are regulated by adding fresh or distilled water, or sea salt containing all the required nutrients. Bacterial inhibitors can also be added to avoid bacterial microflora. The excess biomass from seaweed is collected regularly, and yield is checked to evaluate the proper technique and species (Hirata and Kohirata 1993).

b. **Seaweed cultivation in ponds**: Ponds are the natural body of water where the seawater and river water was mixed, organic and inorganic fertilizers were added if the nutrient contents of the water are low. Sometimes organic fertilizers are introduced during preparation of ponds in the bottom for the seaweed cultivation. By the system of locks water supply, exchange of air and velocity of water flow occurs in ponds. Ponds are covered with plastic mesh to shade cultivated seaweeds e.g. *C. lentillifera*; *Kappaphycus alvarezii*) (Santelices and Doty 1989).

7.7 Utilization of Seaweeds

The microalgae were classified based on pigmentation, the biochemical environment of photosynthetic storage products, association of photosynthetic membrane and other morphological feature (Kilinç et al. 2013) as described above. Seaweeds found to be cultivated/growing throughout the World Ocean and seas and are non-poisonous (Bold and Wynne 1985; Guiry 2009; Lobban and Harrison 1994). These marine microalgae are important economically as well as commercially. Besides this, seaweeds are an important primary producer of the aquatic food chain (Kilinç et al. 2013). Seaweeds are an excellent source of highly active secondary metabolites that can play a significant role in the synthesis of new useful constituents. Various amino acids, certain major and minor nutrients, growth hormones and vitamins are main constituents in seaweed extracts (Mooney and Van Staden 1984). It is widely studied that seaweeds are used as a biofertilizer, soil acclimatizing agent, animal fodder and nutritional supplement for human. Seaweeds also find it importance as pharmaceutical agents due to presence of natural bioactive compounds. These are nothing but a wealth of ocean, a good source of minerals, vitamins, proteins, carbohydrates and fibers (Zamani et al. 2013). The minerals (Mg, K, Cl, S, P) and micronutrients (I, Fe, Zn, Cu, Se, Mo, F, Mn, B, Ni, and Co) are abundant in diverse species of seaweeds. The brown algae have highest number of iodine (Paul et al. 2007). Seaweed contains both water and fat-soluble vitamins. The vitamins (B1, B2, B3, B6, B8, B9, B12, C and E) are present in significant amount in different species of algae. Apart from nutritional support, it has also used against various biological diseases like antimicrobial, antiviral, antifungal, anti-allergic, anticoagulant, anticancer, antifouling and antioxidant activities (Baweja et al. 2009 and Sharma 2014).

Approximately 66% total harvested seaweeds are used foodstuffs, and nearly 100 species were used for phycocolloid production (Zemke-White and Ohno 1999). Due to presence of abundant quantity of minerals and trace elements, seaweeds are used as food material. They have large concentration of protein, minerals, carbohydrates, vitamins and lipids which include polyunsaturated fatty acids (PUFA) and low caloric value which appealing them for human consumption (García-Sartal et al. 2011). These microalgae are the major source of Phycocolloid such as alginate; agar carrageenan, mannitol etc. are used in the food industry. The alginate polysaccharide is most common component of the cell wall of brown algae (*Ecklonia, Undaria*, *Turbinaria* and *Sargassum*) (Kaliaperumal 2003). Alginate contains α-L-guluronic (G) acid and β-D-Mannuronic acid (M) linked together by 1,4-linkage in G-G, M-M and G-M subunits (Renn 1997).

7.8 Potential Applications of Seaweed

Seaweeds prominently find its importance in various industries with multifarious products with potential applications as shown in Fig. 7.4. The potential utilities of seaweeds in different industries provide excellent opportunities for biobusiness as discussed below:

7.8.1 Seaweeds in the Food Industry

Seaweed found a wide spectrum in the food industry. The alginate produced by seaweed used as a gelling agent in the food manufacturing to enhance the viscosity of aqueous solution and form the gel that does not melt when heated. It is also used in ice cream and dairy products as a stabilizers, in soft drinks, forth producer in beer, clarifying agent in wines, increase the viscosity of fruit juices, cheese, syrups, chocolate milk, pudding and meat products (García-Sartal et al. 2011; Harrison

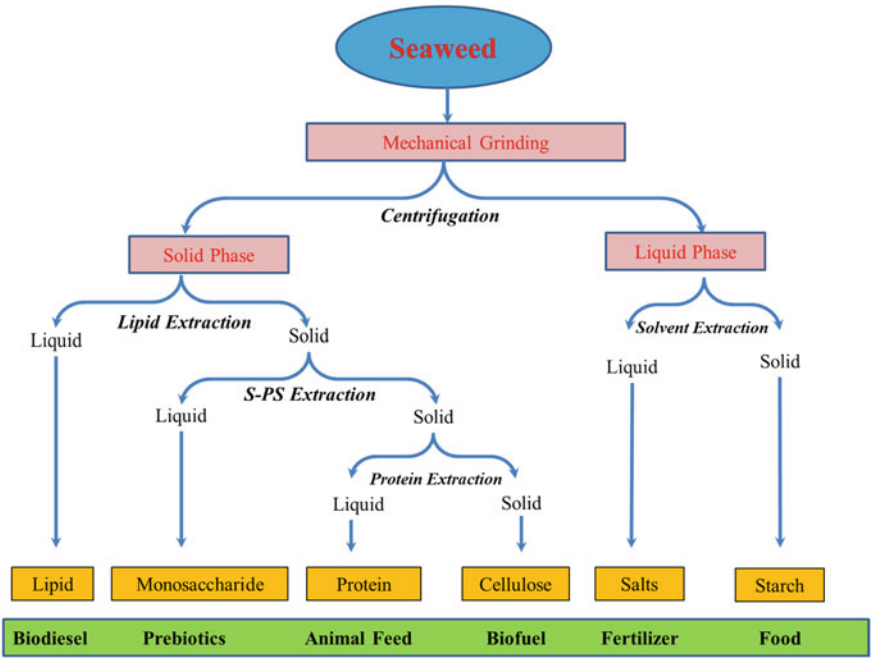

Fig. 7.4 Seaweed a biorefinery for biobusiness with multifarious products for different industries

2008; Johnson et al. 2017; Peteiro et al. 2014). Agar is the major cell-wall constituent of a group of red seaweeds, namely *Gracilaria, Gelidium, Pterocladia* and *Ahnfeltia* out of them *Gelidium* and *Gracilaria* are predominant species used in industry (Bixler and Porse 2010). The agar is used in the food industry because of its property to dissolve in hot water and make a gel. The advantage of agar concerning other gelling compounds (gelatin gels) is that the food products obtained using agar can be stored at room temperature at hot climates (McHug 2003). Carrageenan is obtained from red seaweeds, mainly *Kappaphy cusalvarezii* and *Eucheuma denticulatum.* Denmark, Ireland, Canada, China, Japan and Mozambique are the primary producers of Carrageenan (Dhargalkar and verlecar 2009). Mannitol, important sugar alcohol obtained from brown seaweeds (*Fucus* sp., *Laminaria hyperborean, Sargassum* sp., *Bifurcaria brassiformis*),extracted by using the 10–15% HCl solution and used in making sugar-free products for diabetic persons (Kaliaperumal 2003).

These also act as source of numerous pigments, enzymes, fatty acids, fibers, proteins and vitamins. New food which contains natural ingredients from algae is incorporated in food diets to increase the nutritional value. For example *Chlorella vulgaris* is used as a coloring ingredient in butter cookies (Gouveia et al. 2007). *Spirulina* found its importance as food supplements due to the presence of proteins, fatty acids, fibers, vitamins and microelements (Metting 1996; Liang et al. 2004). In China, *Spirulina* is used in many edible products like noodles, beer, bread, biscuits, drinks, candy and tea (Liang et al. 2004). Presence of pigments, demand of organic, natural and healthy products also makes seaweeds as effective coloring agents in food industry (Batista et al. 2016). Both Chlorophyll a and b also used as food supplement as it increases the blood hemoglobin level, cell reparation capacity and improve growth of cell (Harun et al. 2010). It is also extensively used in making the jams, candy, jelly being allowed in the European Union under food additive code E-140 (Chattopadhyay et al. 2008). Carotenoids such as β-carotenoid, Astaxanthin and lutein are used as a food coloring agent to enhance the color of the product (García-Sartal et al. 2011). Phycobiliproteins, phycoerythrin and phycocyanin are used in the food processing. Phycocyanin collected from cyanobacterium *Spirulina platensis* used in confectionary (Perez-Garcia et al. 2011). A small dosage of phycocyanin has confirmed to prevent the tumor formation in rats (Bealy et al. 1993). Microalgae are a good source of fatty acids. They contain linolenic acid and linoleic acid that act as a precursor of some ω-3 and ω-6 fatty acids, essential for human, used in food and pharmaceutical industries (Chen and Jiang 2001).

7.8.2 Seaweed in Agriculture

Seaweeds are widely used in agriculture as a nutrient supplement and biofertilizer for crops (Khan et al. 2009; Carigie 2010). Research showed that seaweed such as *Ascophyllum, Fucus* are either used directly or in the form of compost, fertilizer or organic materials, fabricating more aired and soothed soils with greater moisture

holding capacity (Carigie 2010). Biointoxicating products from marine microalgae are extracted by various methods. Most commonly, extraction is done chemically by using water, acids or physically by mechanically disrupting the seaweed at low temperature by milling or by using microwave radiations (Sharma et al. 2014). It is reported that these extracts act as stimulants in order to promote plant growth under normal and stressed conditions. Extraction is done mostly by autoclaving it in distilled water (Kumar and Sahoo 2011), or in alkali solutions (Fan et al. 1993). These extracts, stimulates seed germination and root development, also enhance the frost resistance, nutrient uptake and having antifungal and antibacterial properties against certain phytopathogenic fungi (Farid et al. 2009), bacteria (Alves et al. 2016) insect and other pests (Asha et al. 2012). These also used as bioagents for repair of plant growth under stress conditions (Pacholczak et al. 2012).

Microalgae also provide an alternative solution to environment pollution caused by the wide-ranging use of chemical fertilizers. Seaweeds as anorganic fertilizers improve the quality of soil in crop field, nutrients level (N, P and K) and vital minerals (Badar et al. 2015; Mirparsa et al. 2016). According to a report (Thivy 1964), some brown algae used as soil fertilizer due to their rich alginate concentration, which acts as additional organic matter soil microorganisms. Zinc is essential micronutrient in the seaweeds responsible for great plant biomass (Tuhy et al. 2015). Liquid seaweed fertilizer (commonly used in horticulture and agriculture) is the next generation of natural organic fertilizers, having higher nutrients concentration leads to faster seed generation, higher crop yield and enhanced pathogenic resistance (Sathya et al. 2010; Ciepiela et al. 2016). Temple and Bomke (1988) reported positive impact of freshwater kelp (*Macrocystis integrifolia*) on crop growth and nutritional response. The microalgae cocktail released high amount of organic matter and nutrients (NH_4^-, NO_3^- and NO_2^- and phosphate). Certain growth regulators (auxins, gibberlines and cytokinins) present in seaweed extracts and improve the texture, flavor and productivity of vegetables, fruits and other crops (Divya et al. 2015). Seaweed extracts having antioxidant activity and thus improve the damaging effects of scarcity and stress on the *Triticum aestivum* and *Sargassum* directly by stimulating the oxidative system or also by producing essential phytohormones and micronutrients (Kasim et al. 2015). High amount of secondary metabolites (terpenes, lipid, steroid) and aromatic compounds(acetogenins) amino acid, phlorotannins, bioactive compounds (antimicrobial) and other polymeric substances were also produced by seaweed (Farid et al. 2009; Mendes et al. 2010; Asha et al. 2012; Paul 2014; Perez et al. 2016). Seaweeds are capable of eliminating heavy metals, "green synthesis" of iron nanoparticle with brown seaweeds successful used in bioremediation by El-Kassas et al. (2016).

7.8.3 Seaweeds in Pharmaceuticals

Red algae (*Aghardhiellatenera* and *Nothogeniafastigitata*) contain some polysaccharides sulfonates having antiviral properties and helps in cure of various human

infectious diseases (Smit 2004). A xylomannan sulfate from *Nothogenia fastigitata* shows antiviral activities against HIV and Herpes simplex virus type 1 and 2 (Kolender et al. 1995). These polysaccharides became active as defense mechanism when viral RNA starts replication after its attachment to surface of the cell (De Clercq 2000). These antiviral polysaccharides have very low cytotoxic activities, which is an important criteria of any antiviral agent (De Clercq 1996). Fucoidan, a complex brown algal polysaccharide, has antiviral activity toward RSV (Malhotra et al. 2003), HIV and HSV type 1 and 2 viruses (Ponce et al. 2003). It prevents the binding of viral genome to host's cell wall (Baba et al. 1988).

Other seaweeds, namely *Corallina* sp., *Sargassum* sp. and *Hypneacharoides,* also showed high antiviral response against HSV type 1 and 2 and a low level of cytotoxicity (Zhu et al. 2003). Macroalgae have interestingly halogenated compounds like alcohols, haloforms, halogenated alkenes, hydroquinones and terpenoids which have antibiotic activities (Lincoln et al. 1991). Halogenated furanone or fimbrolide belongs to class lactones from *Delisea pulchra* are the potent antibacterial agent and effective against the breakdown of the *Pseudomonas aeruginosa* mucoid alginate in the lungs of patients suffering from cystic fibrosis (Hoiby 2002). Red, green and brown algae contain lectins with hemagglutinating properties (Shanmugam et al. 2002). Lectins are useful in examining cell biding patterns in lectinosorbent assay (Wu et al. 1998). Seaweeds have an anticancerous effect on the human colon (Moussavou et al. 2014). The amino acid, fatty acid and vitamins content of red algae (*Gracilaria*) is high and thus play an important part in the defense against tissue damage wound-healing activities and a noticeable influence on the serum marker enzyme and hepatoprotective activity. *Turbinaria* sp. has secondary metabolites which contain antioxidant activity attributed from DPPH radical scavenging ability. *Terbenaria conoides* is a protectant against gastric ulcers in rats (Boonchum et al. 2012). Green seaweed, namely *Cladophora glomerata* red seaweed *Gracilariacorticata,* and brown seaweeds *Sargassum wightii,* shows antifungal activities against *Aspergillusniger, Aspergillus flavus, Mucor indicus and Saccharomyces cerevisiae* (Mansuya et al. 2010). Alginate extracted from seaweed has used in wound healing. As seaweeds are plants of the unique structure and biochemical composition, they are used for multipurpose requirements.

7.8.4 Seaweeds in the Chemical Industry

Like as living organisms seaweeds synthesized, the primary and secondary metabolites to maintaining their lives. Moreover, in marine environment conditions are multifaceted as there is high salinity, varied temperature conditions, large nutrients subtleties, and strong UV sunlight exposure. These conditions force the microalgae to produces the primary and secondary metabolite with specific chemical and physical properties which make them survive in tough conditions. To protect them to UV radiations, they produce phenolic compounds that possess free radical scavenging properties. Among various seaweeds, brown algae have the

highest polyphenol known as phlorotannins (Baweja et al. 2016). Marine microalgae are one of the potent substitutes of producing energy. This is due to the highest carbon content, rapid growth rate and high photosynthetic efficiency (Roesijadi et al. 2010). Chemical like agar and alginate is produced from marine microalgae. Alginate is used to improve the quality of paper texture and reactive base in reactive dye printing of textiles. Alginate used as stabilizers and emulsifier in food and pharmaceutical industries. It also helps in thickening of rubber (Krishnamurthy 2005). Another example is agar-agar which is obtained from red seaweed, namely *Gelidium, Gracilaria,* etc. and also used as a solid substrate for the growth of bacteria and fungi. The highest quality of agar, which is called as agarose, obtained from red algae family *Gelidiaceae* (Sumedha et al. 2016). Carrageenan is sulfate polysaccharides mainly obtained from red seaweeds. They are mostly edible and used as an emulsion stabilizer, film formers, and hair conditioning agent as well as in the pharmaceutical industry in drug delivery, tissue engineering and antivirals and food industries mainly in dairy products. They have gelling properties (McKim 2014). Green algae also have unusual polypeptides called as kahalalides (Hamann et al. 1996). Additionally, fucoidan from *Fucusvesiculosus* reduces HeLa human cervical tumor growth (Mathew et al. 2017). Many types of biologically active polysaccharides are present in the seaweeds. The sulfate polysaccharide porphyrin is useful as a skin whitening agent for the cosmetic industry. Carotenoids are a great source of pigments, as they function as antioxidants. Tocopherols used as food preservative sunscreens and cosmetics. Phycobiliproteins especially phycocyanin used as a dye (Salehi et al. 2019).

7.9 Sustainable Biomass for Multiple Products

About 25 million tons of macroalgal biomass is harvested annually worldwide, with the massive production, i.e., 95% in Asia. The $5 bn of total Ginazalez seaweed market ($5.6 bn) is used for food products or human consumption and the rest for animal feeds, fertilizer, pharmaceuticals and cosmetics (Beacham et al. 2019). Many waste products or biomass is generated during the processing of algae to any kind of products, which are usually disposed (Uju et al. 2015). Nowadays, there is a need to focus on a no-waste production system for sustainable bioeconomy. Biorefinery (integration of biomass to value-added products and energy) is a step forward in this path and helps in the reduction of adverse effect on the environment and by adding more economical and environmental benefits products (Bikker et al. 2016). Sustainable utilization of seaweed biomass or products is currently challenging, as it is a relatively young and less explored practice of aquaculture. Seaweed cultivation has been exponentially improved worldwide during the past 50 years, and it is silently increasing (FAO 2016b). Seaweed sustainability depends solely on the region of cultivation. Utilization of processed waste remained to add value to the biomass; it is an environmentally friendly process, enhanced biomass alteration efficiency by dropping requirements for raw material. Utilization of

biomass gives more economical benefits, advanced economic constancy and novelties allow developing new biotechnologies (Tiwari and Troy 2015). With increasing interest in seaweed cultivation and utilization, a number of new processing technologies have been emerged out, which increases biomass the conversion efficiency for co-production of high-value speciality and commodity chemicals (Balina et al. 2017).

7.10 Pros and Cons of Seaweed Farming

Aquaculture/seaweed farming is a new emerging opportunity toward sustainability, and economic activity used to convert natural aquatic resources into value-added products for society. It may be having some positive as well as negative impacts on biodiversity.

7.10.1 Pros Associated with Seaweed

- ***Source of food and nutrition***

Seaweed is used as a source of food for centuries. It is mainly consumed in Asian countries like Japan, Korea, China, Indonesia, etc. and used as a traditional source of food. Its nutritional values vary with type and specie. Most of the seaweed is an inorganic source of nutrients like potassium, nitrates, phosphate, etc. (Forster and Radulovich 2015). It is also rich in protein, lipids, vitamins (Cherry et al. 2019). Vitamins content in all types of seaweed is almost the same accept the concentration of vitamins B_{12}, B_1, pantothenic acid, folic and folinic acid are more in green and red seaweed while less in brown seaweed. On the other hand, brown seaweeds are a rich source of organic iodine (Kilinç et al. 2013).

- ***Grow rapidly and cost-effective***

Some seaweeds like *Macrocystispyrifera* and *Laminaria japonica*are fast-growing and have the tendency to give multiple crops per year. As they are grown in the coastal region and these region is rich in nutrition that is why fertilizers are not needed, which directly decreases the cost of its production (Forster and Radulovich 2015). Seaweed is least expensive for the preparation of new chemicals as compared to other invertebrates (Ireland et al. 1993).

- ***Environmental impact***

As we know that seaweeds are the primary producer in the food chain and they produce their food using photosynthesis. They use carbon dioxide during the process of photosynthesis. In this way, it gives positive contribution by mean of

carbon sequestration (Hasselstrom et al. 2018). Seaweed farming helps in improving the soil quality as it can be used as fertilizers (Duarte et al. 2017).

7.10.2 Cons Associated with Seaweed

The seaweed farming is very recent as compared to the agriculture farming that is why its operation and mechanization is not very much efficient as compared to the traditionally used agriculture farming (Forster and Radulovich 2015). Some seaweeds are toxic as they can store heavy metals like arsenic, mercury, lead, etc. Seaweeds also contain polysaccharide like agar, xylans, etc. which inhibit the digestion of protein. Despite its nutritional values, it is not recommended to any allergy-risk person as it produces more allergic response as compared to any other edible marine products. Ogo-nori an example of seaweed which causes anaphylactic shock in allergy-risk people (Noguchi et al. 1994). Major cons associated with are as follows (Fleurence et al. 2012):

- Real market applications are different than theoretical studies and needs association between the legislative body of market sectors.
- Scientific and technological challenges occur as it is a new and not immature technology.
- Climate change and certain policies affect availability, quality and energy density of raw materials and make it difficult to find investment capital.
- High class standard are applied to bio-based products than traditional ones.

7.11 Conclusion

Seaweeds have received increasing global attention as a useful potential ingredient in various food, feed, cosmetics and pharmaceutical products or as raw material for chemical, biomaterials and energetic products. The key problems associated with seaweed industry are scarcity of raw material as a result of overexploitation, and poor quality of raw material. Besides these, problems of manpower shortages during harvesting, transplanting season, availability of suitable improved technologies for enhancing the yield as well as the quality of finished product have been other serious bottlenecks in promoting seaweed farming. Efforts are required to enhance seaweed production by improving harvesting techniques, elimination of contending species, designing of artificial habitats and seeding. Extensive sincere efforts are also needed to recognize/identify potential commercial species and suitable sites for large-scale cultivation of seaweeds. Value-added quality products are required to be developed as per the market demand backed-up by strong and effective marketing strategies to popularize the seaweed derived products among the users and continuous improvement of the product quality. The need of the

present time is to train, encourage and promote coastal fishermen as well as common people to opt for seaweed farming with continuous technological inputs and assured marketing. Based on the information provided in this chapter, it can be concluded that seaweed farming or aquaculture concept can significantly improve the economic status of farmers if the needed appropriate technological interventions are applied for development of useful value-added products. Better understanding of the overall technological processes and pathways is also required to make seaweed cultivation a safe and economical source of biomass for local farmers, industries, region and nation as a whole.

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Chapter 8
Utilization of Weed Plants for Biochemicals and Bioactive Compounds Production

Vijay Kumar, Virender Kumar, Ranjit G. Gurav, Yung-Hun Yang, and Shashi Kant Bhatia

8.1 Introduction

Weeds are undesired plants and dominant competitors of desired agriculture, horticulture, or other ornamental plants. Weed plants have high vigor, persistence, produce more seeds, have high seed dormancy, and have the ability to spread quickly (Ekwealor et al. 2019). Apart from the agricultural land, weeds plants grow in almost every kind of niches such as forest, wasteland, roadside, industrial wasteland, construction sites, and even other manmade or natural extreme conditions (Priya et al. 2014). Among weed plants, the alien or invasive weeds are of considerable scientific and social interest. Invasive weeds are non-native plants which spread to new regions, may be different continent, and overpower the flora of new location rapidly (Jaggi et al. 2017). Invasive weed plants outcast or overshadow the native plants by various mechanisms such as by competing for nutrients efficiently, by allelopathic mechanism, and with more successful reproductive

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strategies. In addition, invasive weeds usually have a high tolerance to the abiotic/biotic stresses and edaphic conditions (Bajwa et al. 2016; Ekwealor et al. 2019). Probably, that is why in his famous book "Defense of Nature's Most Unloved Plants" Richard Mabey, a famous writer has described the weeds plants with many names such as the notorious, the trespassers, the vegetable guerillas, the outlaws, and the botanical fifth columnists (Mabey 2017).

Weed plants are obnoxious causing health, agriculture, and biodiversity hazards. The devastating effect of alien weeds can be realized by an example of *Hypericum perforatum* L. (Klamath weed), which is native to Europe and had spread in California, near river Klamath. This weed spread rapidly over two million acres of land in California during World War II and pose serious challenges to land productivity and causes weight loss to cattle and sheep. The problem was so serious that some beetles were imported from Australia to control this weed as they feed on it. After an effort of 10 years, the Klamath weed could be controlled and reduced to >99%. The effect on the increase in the land values, and cattle and sheep weight loss was ceased as results. Many other weeds and alien weeds showing allelopathy are noxious to humans and animals. For example, *Parthenium* weed causes skin allergy, irritation, and dermatitis in the peoples (Sharma and Sethuraman 2007). Another noxious weed *Lantana* causes hepatotoxicity and various histopathological complications in animals (Kumar et al. 2016). Apart from the toxicity to humans and animals, alien weeds have a serious impact on biodiversity and ecosystems, including agriculture, forestry, and agro-forestry (Jaggi et al. 2017). The assessment of economic loss due to weeds and alien weeds to agriculture and overall development is difficult but estimated in various ranges from less than 0.001 to 12% of total GDP for different countries (Marbuah et al. 2014). In India, every year weeds cause estimated food loss of around 20 metric ton (Priya et al. 2014) and around 11 billion USD loss to major crops (Gharde et al. 2018).

Apart from being a bad boy, weeds, however, also have many important properties that can be beneficial to fulfill the humans and societal needs (Ekwealor et al. 2019). Weed plants biomass are rich and cost-effective source of lignocellulosic biomass and can be used for the production of bioenergy, biochemicals, and biomaterials (Ciesielczuka et al. 2016). The important biochemicals from weeds can be harnessed for new applications in pharmaceuticals, medicine, and agriculture. Some weeds plants produce chemicals that repel insects; thus, they can be explored for their applications as the larvicidal and insecticidal. Furthermore, many weeds can be used for phytoremediation purposes (Raj and Syriac 2016). Certain weeds, especially, seaweeds can be explored as feed and food with nutritional value and nutraceutical benefits (Charoensiddhi et al. 2017). This chapter focuses on bioactive and biochemicals production from some weed plants using direct extraction processes and also the utilization of the weed biomass as a carbon source for microbial fermentation. For the convenience of readers, the definition of few keywords discussed in this chapter is mentioned below:

Weeds: Weeds are plants that are undesired for humans, animals, and agriculture perspective. They cause competition for economically important plants or agricultural crops. They may also pose a challenge to biodiversity and causes health hazards.

Aquatic weeds: Similar to the terrestrial weeds, the unwanted and undesirable plants that grow in water are called aquatic weeds. They may be very problematic for water bodies and fish farming.

Alien or invasive weeds: These are the plants that are non-native or non-indigenous origin. They have origin in a different geographical region and have migrated to a new location through various means. They usually destroy the ecosystem by eliminating the native flora through competition.

Seaweeds: Seaweeds refer to the many species of microalgae, especially green (*Chlorophyta*), brown (*Phaeophyta*), and red (*Rhodophyta*) algae. Unlike the terrestrial weeds, they are important for the marine ecosystem as well as for human beings. They play a crucial role in carbon capturing and global oxygen production.

Allelopathy: A strategy of weed plants to release some chemical substances that are generally toxic and inhibitory for the other plants. This phenomenon is a key for successful competition and is called allelopathy. The weeds release these photochemical called allelochemicals through various means such as volatiles and root exudates.

Bioactive molecules: A substance or a chemical compound that showed a characteristic biological activity. The biological activity may have beneficial or adverse effects on the biological system. However, in this chapter, the bioactive is the compounds that showed beneficial activity as per the human perspective, for example, antimicrobial, inhibiting the growth of pathogenic bacteria and fungi, antitumor, insecticidal, pesticidal, larvicidal, and nematicidal effect.

Nutraceutical: It is a product that may be used as food or part of the food (dietary supplements) and provides medical and health benefits.

8.2 Some Weed Plants and Their Potential Utilization

Rapid globalization, industrialization, and the increasing human population have put tremendous pressure on agriculture and forest land. Overburden and selective cultivation of high yielding crops, human has declared a number of plants as weeds, as they are not desired. However, taxonomically and botanically, the term weed has no scientific significance. Globalization has also circulated the flora from their native homeland to a new location, where, some of them have found very suitable conditions for growth. Soon many of the plants such as *Lantana*, *Parthenium*, *Salvinia*, *Eichhornia*, and many more have become pandemic and declared as invasive weeds. For example, *Lantana* is native to the tropical area of Africa and America but now covers a vast area in the Australian Pacific region and India. *Parthenium* is native to North America but has invaded a significant area in India and other old world countries. *Salvinia molesta* and *Eichhornia crassipes* are

original inhabitants of South America but had spread across the warm climate in other parts of the world. A long list of various invasive weeds is compiled and updated in various databases across the world. To discuss the each and every weed plants and invasive weeds is not in the scope of this book chapter, so a concise table of some of the weed plants is presented for the reader convenience (Table 8.1).

Table 8.1 Some common weed plants and their distribution across the globe

Weed plant	Scientific name	Continents/region
Lantana	*Lantana* spp.	Part of America, Europe, Africa, Asia, and Oceania
Carrot grass, congress grass	*Parthenium hysterophorus*	Central America, India, Australia, and parts of Africa
Water hyacinth	*Eichhornia crassipes*	North America, Europe, Asia, Australia, Africa, and New Zealand
Giant salvinia, kariba weed	*Salvinia molesta*	Tropical and subtropical areas across the globe
Siam weed, Christmas bush, devil weed	*Chromolaena odorata*	America, Tropical Asia, West Africa, and parts of Australia
Giant sensitive plant	*Mimosa invisa*	South America, USA, some part of Asia
Miscanthus, silvergrass	*Miscanthus* spp.	Africa, Eurasia, and Pacific island
Switch grass	*Panicum virgatum*	America and some part of Asia
Chick weed, winter weed	*Stellaria media*	Asia, Europe, and North America
Alligator weed	*Alternanthera philoxeroides*	South America, USA, and China
Cogon grass	*Imperata cylindrica*	Tropical and subtropical Asia, Australia, Africa, some part of Europe, and America
Napier grass, elephant grass	*Pennisetum purpureum*	Africa, Central and South America, tropical parts of Asia, and Australia
Vetiver grass	*Vetiveria zizanioides*	India and other parts of Asia
Bitter vine, American rope	*Mikania micrantha*	America, India, and tropical part of world
Pink morning glory	*Ipomoea carnea*	Asia, Africa, South America, and Oceania
Amaranth, pig weed	*Amaranthus* sp.	Every continent and tropical lowlands to the Himalayas
Indian abutilon, Indian mallow	*Abutilon indicum*	Tropic and subtropical regions and India
Kamlath weed	*Hypericum perforatum*	Temperate parts of Europe, Asia, and America

(continued)

Table 8.1 (continued)

Weed plant	Scientific name	Continents/region
Buffalo weed, giant ragweed	*Ambrosia trifida*	North America and some part of Europe and Asia
Crofton weed, sticky snakeroot	*Ageratina adenophora*	America, Asia, Australia, and many tropical and subtropical countries including India
Goat weed, white weed	*Ageratum conyzoides*	America, Africa, Australia, Southeast Asia, and India
Blue weed, floss flower	*Ageratum houstonianum*	Central America, North America, Africa, Asia, Europe, and Oceania
Apāmārga, chaff-flower	*Achyranthes aspera*	Tropical region of America, Africa, Asia, and Australia

Source Wikipedia; Invasive.org (https://www.invasive.org/species/weeds.cfm); Global Invasive Species Database (http://www.iucngisd.org/gisd/about.php); National Invasive Species Information Center (NISIC) (https://www.invasivespeciesinfo.gov/)

8.3 Potential Utilization of Weed Plants for Various Applications

The weeds biomass is consisting of lignocellulosic materials and it represents a huge reservoir of feedstock for the production of bioenergy, biomaterials, and bioethanol. Weed plants are blessed with lower water and nutrients demands, therefore, lignocellulose content in such plants is an attractive feedstock. A detailed description of the chemical composition of lignocellulose biomass including the cellulose, hemicellulose, lignin, and ash contents of some weed plants was reported and discussed (Premjet 2018). The weeds biomass as a source of lignocellulose needs no extra effort that they itself grow in a large area without any effort. Moreover, a big problem of agriculture and horticulture will be solved on the other hand. Weed plants management is an active area of research and needs more rigorous scientific intervention. The weeds biomass can be a rich source of cellulosic/hemicellulose, lignin, proteins, and amino acids, lipids, and fatty acids, and the plethora of secondary metabolites (Fig. 8.1).

The proteins from weeds may be a good source for animal feed and extraction of amino acids and nutraceuticals. The lipids and fatty acids can be processed to obtain volatile fatty acids for bioenergy and food applications. The fourth category and secondary metabolites have tremendous newer possibilities. The weeds have been used in the past and being explored to find the bioactive molecules with newer bioactivity. A range of aromatics, alkaloids, terpenoids, steroids, triterpenoids, glycosides, and much more can be obtained from many weed plants. Most of these commercially important compounds can be extracted using the mechanical, enzymatic treatment, and solvent extraction processes or by combinations of these methods. Weed biomass can also be used as a raw material for microbial fermentation to produce various biochemicals. Here, we have discussed various

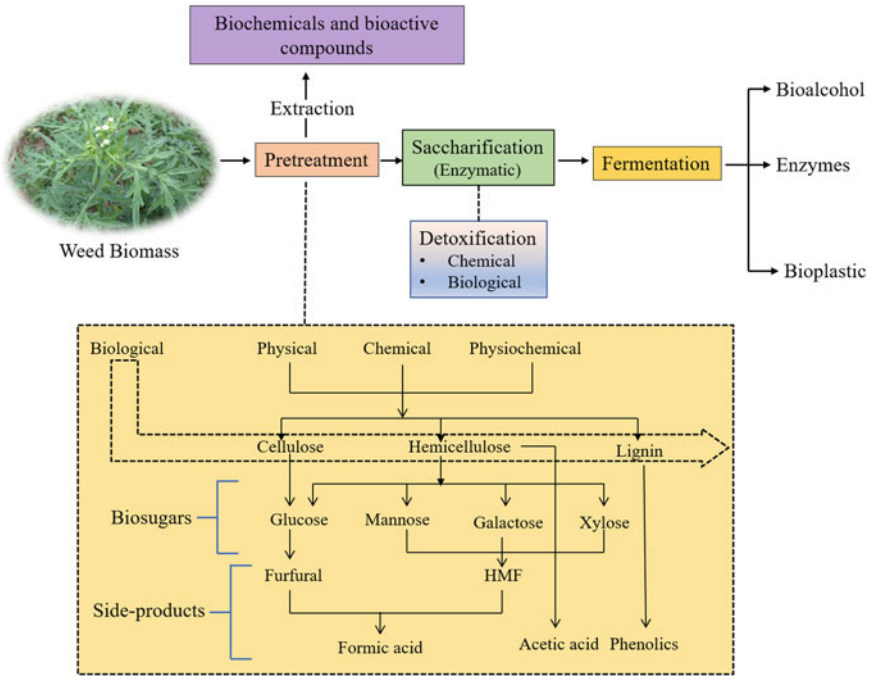


Fig. 8.1 Overall process design for weed biomass pretreatment and byproduct production

products (bioactive compounds and nutraceuticals) extracted from weed biomass and biochemicals produced through weed biomass fermentation.

8.3.1 Bioactive Compounds Extracted from Some Weed Plants

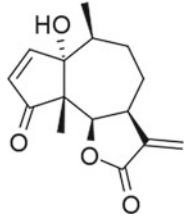
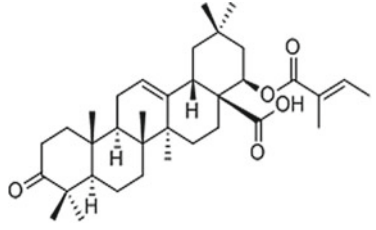
Weed biomass can be used for a number of productive purposes but this section is solely focused on the bioactive compounds from various weed plants. The process of finding a bioactive molecule is a tedious process and requires multidisciplinary efforts from the researcher of plant sciences, natural products chemistry, biotechnology, and bioinformatics. Basic techniques such as solvent extraction, thin layer, and column chromatography along with high-end analytical techniques such as UV-visible and infra-red spectroscopy, high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) are required to purify, identify, characterize, and structural determination of bioactive compounds. Several solvents, i.e., water, methanol, ethanol, hexane, ethyl acetate, acetone, hexane, chloroform, and dimethylformamide (DMF), etc., or their combination are used for the extraction of bioactive

molecules from various parts of a plant. These solvents are selected for the extraction based on the polarity of bioactive compounds. Further, multiple solvents are also used in a sequential order to purify the bioactive compounds (Altemimi et al. 2017). A number of modifications of traditional solvent extraction using physical principles such as chromatography, soxhlet extractor, ultrasonic assisted solvent extraction, and supercritical fluid extraction have been employed for fast and efficient extraction and recovery of bioactive compounds as reviewed elsewhere (Zhang et al. 2018). The bioactivity may be followed by the purification of bioactive compounds or may be done before the analytical analysis often called bioactivity guided discovery. Readers who are more interested in the area of natural product discovery and their bioactivity analysis may read some interesting research and review papers elsewhere (Luo et al. 2014; Li and Lou 2017; Nothias et al. 2018; Newman and Cragg 2020) and also presented in Table 8.2.

Due to the advancement in analytical technologies and the need to manage the increasing weeds led to the exploration of the weed plants for potential bioactive molecules. A number of research groups in the last two decades have explored the weed plants and invasive weeds for new and already known bioactives and their potential bioactivities (Table 8.2). For example, the weed *Parthenium hysterophorus* was an allergenic, irritant, and causes dermatitis, especially during summers (Sharma and Sethuraman 2007). The main bioactive molecule in *P. hysterophorus* is parthenin, a sesquiterpene lactone. This compound was found to have pesticidal and insecticidal potential (Datta and Saxena 2001; Reddy et al. 2018). *Lantana camara*, a cosmopolitan weed, was found to be a source of a number of secondary metabolites including lantadenes A and latadenes B showing anticancerous activity (Sharma et al. 2008; Grace-Lynn et al. 2012). Furthermore, thus, weed have various pentacyclic triterpenoids such as pomolic acid, lantanolic acid, lantoic acid, camarin, lantacin, camarinin, ursolic acid, and lancamarolide with nematicidal property (Begum et al. 2008, 2015). Water hyacinth (*E. crassipes*) is an aquatic weed and produces a range of alkaloids, terpenoids, phthalate derivatives, propanoid, and phenyl derivatives showing various bioactivities (Aboul-Enein et al. 2011, 2014). Another aquatic weed *S. molesta* (kareeba weed) extract has various glycosides and abietane diterpenes such as salviniol, salviniside I, and salviniside II with anticancerous activity (Choudhary et al. 2008; Li et al. 2013a).

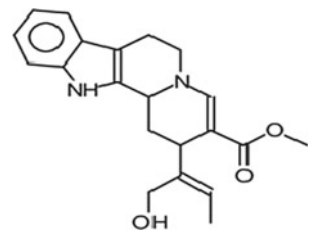
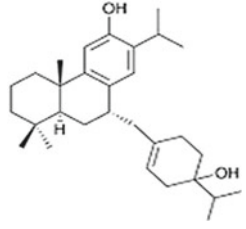
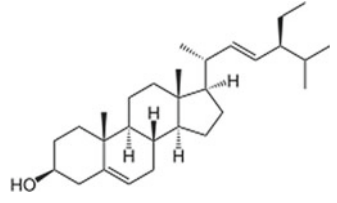
S. molesta is a source of numerous phenolics and hydroxyacids such as methyl benzoate, hypogallic acid, caffeic acid, paeoniflorin, and pikuroside (Choudhary et al. 2008). Similarly, some other weeds, i.e., *Miscanthus*, *Mikania,* and *Hypericum* are also produced various aromatics and aromatic hydoxyacids (Villaverde et al. 2008; Brosse et al. 2012; Li et al. 2013b; Mehta 2012). Hydroxyacids have various bioactivities, i.e., antimicrobial, antiaging, and anti-inflammatory, therefore, are used in various direct applications as well as for the synthesis of other important organic compounds (Bhalla et al. 2014). Some important aromatic hydroxyl acids such as *p*-hydroxybezoic acid, vanillic acid, and mandelic acid have been synthesized by enzymatic transformations on the rationale to provide an alternative route to chemical synthesis (Kumar and Bhalla 2013; Kumar et al. 2015a; Bhalla et al. 2016; Bhatia et al. 2013). Thus, the weed plants

Table 8.2 Bioactive compounds extracted from some weed plants

Weed plant	Extraction methods	Bioactive molecules	Chemical structure[a]	Bioactivity	References
Parthenium hysterophorus	Solvent extraction	Parthenin		Pesticidal, insecticidal	Datta and Saxena, (2001) and Reddy et al. (2018)
Lantana camara	Methanol extraction followed by solute partitioning	Lantadenes	 Lantadene A	Antitumor, antioxidant	Sharma et al. (2008) and Grace-Lynn et al. (2012)
		Pentacyclic triterpenoids		Nematicidal	Begum et al. (2008, 2015)

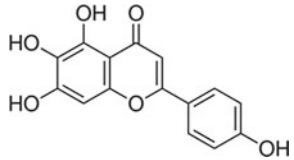
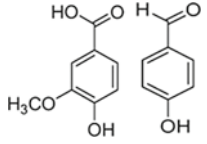
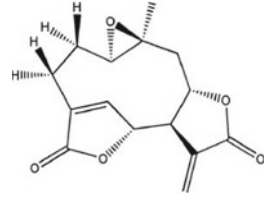
(continued)

Table 8.2 (continued)

Weed plant	Extraction methods	Bioactive molecules	Chemical structure[a]	Bioactivity	References
Eichhornia crassipes	Methanol extraction using rotary evaporator followed by fractionation in mixture of hexane/ethyl acetate	Alkaloids, terpenoids, phthalate derivatives, propanoid, and phenyl derivatives	 Alkaloid derivatives	Antimicrobial, antioxidant, antitumor	Aboul-Enein et al. (2011, 2014)
Salvinia molesta	Solvent (Ethanol) extraction	Salviniol, salviniside I, salviniside II, various abietane diterpenes	 Salvinol	Anticancerous	Li et al. (2013a)
Chromolaenaodorata	Methanol extraction followed by fractionation using column chromatography	Stigmasterol, hexacosanol	 Stigmasterol	Larvicidal, insect repellant anti-cholesterolemic effect	Gade et al. (2017) and Ikewuchi and Ikewuchi (2011)

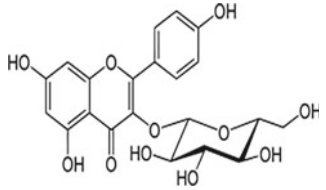
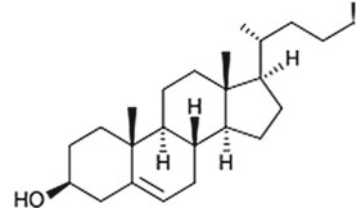
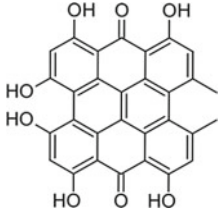
(continued)

Table 8.2 (continued)

Weed plant	Extraction methods	Bioactive molecules	Chemical structure[a]	Bioactivity	References
		Scutellarein tetramethyl ether, stigmasterol, isosakuranetin	 Scutellarein tetramethyl ether	Anti-inflammatory	Pandith et al. (2013)
Miscanthus giganteus	Soxhlet extraction using dichloromethane	Aromatics, sterols, fatty acids, long-chain fatty alcohols	 Vanillic acid, p-hydroxybenzaldehyde	Chemical synthesis	Villaverde et al. (2008) and Brosse et al. (2012)
Mikania micrantha	Ultrasonic-assisted chloroform extraction followed by chromatography	Sesquiterpenoids (deoxymikanolide, dihydroscandenolide, mikanolide, scandenolide, dihydromikanolide) m-methoxy benzoic acid	 Deoxymikanolide	Antibacterial, antifungal	Li et al. (2013b)

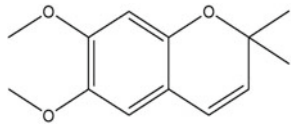
(continued)

Table 8.2 (continued)

Weed plant	Extraction methods	Bioactive molecules	Chemical structure[a]	Bioactivity	References
Ipomoea carnea	Solvent extraction	Alkaloids, secondary metabolites, kaempferol glucoside	 Kaempferol glucoside	Antioxidant, antimicrobial, anticancer, antidiabetic, immunomodulatory	Ambiga et al. (2007) and Srivastava and Shukla (2015)
Stellaria media *Abutilon indicum*	Successive solvent extraction from lower to higher polarity solvent extraction	Phenolics, saponin, flavonoids, steroids (β-Sitosterol) triterpenoids, glycosides, β-Sitosterol	 β-Sitosterol	Anti-obesity larvicidal	Chidrawars et al. (2011) and Rani et al. (2012) and Rahuman et al. (2008)
Hypericum perforatum (L.)	Soxhlet extraction using ethanol at high-pressure with supercritical CO_2	Flavonoids, phenolics, naphthodianthrone, tannins, volatile oil	 Hypercin	Antimicrobial, antiviral, antidepressant	Cossuta et al. (2012) and Mehta (2012)

(continued)

Table 8.2 (continued)

Weed plant	Extraction methods	Bioactive molecules	Chemical structure[a]	Bioactivity	References
Ageratum houstonianum	Solvent extraction, rotary evaporator, and solvent partitioning	Pyrrolizidine alkaloids; agerarin (6,7-dimethoxy-2,2-dimethyl-2H-chromene)	 Agerarin	Antimicrobial, skin wound healing, aquaporin-3 gene expression in keratinocytes	Wiedenfeld and Andrade-Cetto (2001) and Shin et al. (2017)

[a]Weed plants generally produce a number of secondary metabolites. To present the structure of each and every metabolite is beyond the scope of this chapter, so a representative compound or important one are presented in the table

can also be explored more rigorously as a potential source for the economical production of aromatic hydroxyacids.

An anti-cholesterolemic compound stigmasterol was characterized from *Chromolaena odorata* (Ikewuchi and Ikewuchi 2011). Another study reported scutellarein tetramethyl ether from this weed with anti-inflammatory activity (Pandith et al. 2013). The same weed has hexacosanol in addition to stigmasterol and found to have acetylcholinesterase enzyme inhibitory action which led to larvicidal and insect repellant activity (Gade et al. 2017). *Ipomoea* weed was found to be a source of a number of alkaloids and secondary metabolites including kaempferol glucoside and has shown a plethora of bioactivities (Ambiga et al. 2007; Srivastava and Shukla 2015). β-Sitosterol, an important phytosterol found in many plants, has been extracted from *Abutilon indicumi* and *Stellaria media* and showed anti-obesity and larvicidal potential (Rahuman et al. 2008; Chidrawars et al. 2011; Rani et al. 2012). In addition to sitostreol, *Stellaria* has various phenolics, saponin, flavonoids, triterpenoids, glycosides, and anthocynidin (Chidrawars et al. 2011; Rani et al. 2012). A naphthodianthrone, hypericin extracted from *H. perforatum* had shown antidepressant activity in addition to antimicrobial and antiviral bioactivity (Mehta 2012). *Ageratum conyzoides* and *Ageratum houstonianum* are known to produce pyrrolizidine alkaloids (Wiedenfeld and Andrade-Cetto 2001), which cause poisoning and liver lesions to the grazing animals. However, a recent study identified a new compound agerarin (6,7-dimethoxy-2, 2-dimethyl-2H-chromene) from the *A. houstonianum* which are found to up-regulate the aquaporin-3 genes in keratinocytes suggesting for its application in skin disease (Shin et al. 2017). Thus, we see a number of weeds, which are considered as unwanted, useless, or harmful, are also a very useful resource to hunt the bioactive compounds. Therefore, weed plants can be bioprospected for a range of new and known secondary metabolites with a plethora of bioactivities.

Seaweeds are the macroalgae growing in the coastal regions and oceans. On contrary to weed plants, seaweeds are traditionally used since ancient times. In this chapter, we just give an overview of the seaweeds as sources of various important biochemicals. Seaweeds produced various classes of biomolecules such as polysaccharides, proteins, peptides, fatty acids, and various secondary metabolites (Charoensiddhi et al. 2017; Sanjeewa et al. 2018; Admassu et al. 2018; Chen et al. 2018; Tanna and Mishra 2019). Most common examples of the polysaccharides produced by seaweeds are agar, agarose, alginates, carrageenan, laminarin, and fucoidan. Among the agar, agarose, and carrageenan are obtained from various red seaweeds, ulvan is obtained from a green seaweed, whereas various species of brown algae produce laminarin, fucoidan, and alginate (Tanna and Mishra 2019). Apart from these known polymers, the seaweeds houses for a range of new polysaccharides. For example, a new polysaccharide (SPm) consisting of sulfated α-L-rhamnose and a small amount of xylose and glucuronic acid was found in marine green algae *Monostroma angicava* (Liu et al. 2018). A similar kind of sulfated rhamnan obtained from *Monostroma nitidum* was found to have anticoagulant activity (Okamoto et al. 2019). Bioactive peptides from seaweed are one of the promising areas for therapeutics and nutraceutical formulations. Seaweeds

derived peptides have shown various bioactivities such as antihypertensive, antioxidative, and antidiabetics, which are very beneficial for the management of cardiovascular disease and diabetes (Admassu et al. 2018). These bioactive peptides are obtained by disrupting the cells of seaweed by physical and enzymatic methods followed by the digestion of proteins by various proteolytic enzymes. Some important polyunsaturated fatty acid (PUFA), i.e., omega-3 (n-3) and omega-6 (n-6) and fish fatty acid eicosapentaenoic acid are obtained from various seaweeds of Atlantic and tropical seas (Van Ginneken et al. 2011). These PUFAs have many health benefits including the prevention of cardiovascular diseases. Various bioactive compounds showing algicidal activity including several fatty acids were extracted from the *Ulva prolifera* (Sun et al. 2016). An important bromophenol 3-bromo-4, 5-dihydroxybenzaldehyde was extracted form *Polysiphoniamorrowii* and found to have antiviral, antioxidant, and cytoprotective effect (Kim et al. 2011, 2017a). A number of antioxidant metabolites namely taondiol, isoepitaondiol, stypodiol, stypoldione, sargaquinone, and sargaol were obtained from *Taonia atomaria* (Nahas et al. 2007). Antimicrobial bioactive compounds such as peyssonoic acid A and peyssonoic acid B were extracted from the *Peyssonnelia* sp. (Lane et al. 2011). Diterpene sargafuran showing anti-acne activity was found in brown algae *Sargassum macrocarpum* (Kamei et al. 2009). The brown algae, especially the genus *Dictyota* was found to be a huge source of a large number of bioactive compounds belonging to the diterpenes group (Chen et al. 2018).

Overall, it can be concluded that weeds, weather weed plants, or seaweeds are rich source of bioactive compounds. This is the time to look for the large-scale production of desired bioactive from the weed plants. This strategy will be unique for the weed management. It will reduce the dependency on chemical processes and also help in the conservation of medicinal and aromatic flora. A collaborative effort from academia and industry people can pinpoint the desired bioactives, develop the processes, and further commercially produces the bioactives from weeds to meet the societal demands.

8.3.2 Various Nutraceutical Extracted from Weed Biomass

Nutraceutical aims to supplement the desirable food and health components in a concentrated form. It can be elaborated in two forms, i.e., functional food and dietary supplements. Functional food is the enriched or fortified food materials to restore pre-processed nutrient levels to improve the nutritional quantity of nutrient-deficient food or to resolve public health issues (e.g., iodized table salt). In retrospect, the dietary supplement is another class of nutraceutical that includes concentrated forms of food-derived nutrients. Dietary supplements are not exactly food replacements (Mishra et al. 2018). These are taken in addition to daily food for further health benefits. The rising awareness about healthy living among the population has a new group of buyers of nutraceutical products. Therefore, the vast diversity of plants including weeds can be explored for nutraceutical. The weed

plants (terrestrial, aquatic, or sea) have immense potential for usage as a nutraceutical, therapeutic, as well as medicinal purposes (Zhou et al. 2015). The weed plants contain many different bioactive constituents ranging from important amino acids, alkaloids, flavonoids, terpenes, fatty acids, steroids, and organic acids (Mishra et al. 2018). The different parts of weed plants can be processed and used for the treatment of various disorders and to increase immunity as well as for health benefits. For example, *Eclipta alba* (L.) Hassk, a weed plant is used to treat various respiratory infections, throat pain, fever, etc. (Jahan et al. 2014). It is reported to contain bioactive components like organic acids (oleanolic acids), lactones (wedelolactone), as well as luteolin and apigenin—which have potential health benefits upon intake. *Matricaria chamomilla* contains coumerins, glucosides, and caffeic acid and has been reported to have fungistatic, antiseptic, and antiplogistic applications. *Ipomoea hederacea* has been reported to cure inflammations, useful in liver and spleen diseases, and joints pain (Zia-Ul-Haq et al. 2012). Neuroprotective, antimicrobial, antidiabetic, antioxidant, anti-inflammatory, antiulcerogenic, and anticancer activities have been demonstrated in *Portulaca oleracea* due to the presence of flavonoids, alkaloids, polysaccharides, fatty acids, tarpenoids, and sterols (Zhou et al. 2015).

The nutraceutical industry is growing rapidly and a number of products available ranging in the form of isolated nutrients, dietary supplements, herbal products, and processed foods in the form of cereals, soups, and beverages. Thus, in this emerging sector, the weeds plants can also contribute to be utilized and projected as nutritionally enriched foods (Mishra et al. 2018). Overall, in the present scenario, weed plants have a significant role in the nutraceutical industry and it can lead to enhance the farmer's income through the use of undesired weed plants for human health. Some of the important weed plants and their nutraceutical applications have been compiled (Table 8.3).

8.3.3 Conversion of Weed Biomass into Biochemicals Through Fermentation

Weed biomass is mostly composed of lignin, hemicellulose, and cellulose. The cellulose and hemicellulose can be converted into free sugars and used as raw material for the microbial fermentation to produce various biochemicals such as bioalcohols, organic acids, enzymes, and bioplastics (Bhatia and Yang 2017; Bhatia et al. 2019; Jeon et al. 2018). Biomass is recalcitrant and microbes are not able to utilize it directly and need various pretreatment methods (Bhatia et al. 2020). Different pretreatment methods such as physical (milling, chipping, and grinding), chemical (acid, alkali, ionic liquid), physicochemical (steam explosion and CO_2 explosion), and biological methods (enzymatic) have been reported to release free sugars from biomass (Bhatia et al. 2017a). The main aim of pretreatment is to break supramolecular structure of lignin-cellulose-hemicellulose and release free sugars.

Table 8.3 Nutraceutical properties of some weed plants

Weed plant	Bioactive molecules	Nutraceutical use	References
Achyranthes aspera (L.)	Flavonoids, alkaloids, saponins, and triterpenoids	Pneumonia, cough, and kidney stones	Bhosale et al. (2012)
Argemone mexicana (L.)	Alkaoidsberiberi and protopine	Jaundice, cutaneous infection, tumors	Brahmachari et al. (2013)
Eclipta alba (L.) Hassk	Wedelolactone, oleanolic acids, luteolin, and apigenin	Cure diarrhea, throat pain, and to reduce fever	Jahan et al. (2014)
Ipomoea hederacea.(L.) Jacq	Ecdysteriods, steroidal glycosides, aromatic acids, triterpenes, amino acids, organic acids	Cures inflammations, useful in liver and spleen diseases, and pain in joints	Zia-Ul-Haq et al. (2012)
Matricaria chamomilla (L.)	Coumarins, chlorogenic acid and caffeic acid (phenylpropanoids), apigenin, apigenin-7-O-glucoside, luteolin, and luteolin-7-O-glucoside	Fungistatic, antiseptic, and antiplogistic	Singh et al. (2011)
Mimosa pudica (L.)	Alkaloids, mimosine, flavonoids C-glycosides, sterols, terpenoids, tannins, and fatty acids	Antibacterial, antivenom, antifertility, anticonvulsant, antidepressant, and aphrodisiac	Ahmad et al. (2012)
Portulaca oleracea (L.)	Flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols	Neuroprotective, antimicrobial, antidiabetic, antioxidant, anti-inflammatory, antiulcerogenic, and anticancer activities.	Zhou et al. (2015)
Stachytarpheta jamaicensis (L.) Vahl	Alkaloids, carbohydrates, saponins, tannins, and terpenoides	Antimicrobial, antifungal, antioxidant, and antihypertensive	Liew and Yang (2016)
Urtica spp.	Iron, tannins, and polyphenols	Antimicrobial and antioxidant	Kregiel et al. (2018)

During the pretreatment various side products such as furfural, organic acids, and hydroxymethylfurfural get produced which affects the microbial growth and fermentation by causing intracellular acidification and accumulation of reactive oxygen species (ROS) (Bhatia et al. 2016, 2017b; Song et al. 2017). It is important to remove these inhibitory compounds from the hydrolysate before using it as a carbon source for microbial fermentation. Various physical (evaporation, membrane filtration), chemical (resin exchange, active carbon adsorption), and biological (enzymatic and microbial) methods have been reported for the removal of inhibitor compounds (Bhatia et al. 2020). A brief outline of the whole process of weed

biomass to biochemical production using various physical, chemical, and biological treatments is presented (Fig. 8.1).

Biomass of several weed plants is used as a substrate for microbial fermentation after suitable pretreatment. For example, *Hydrodictyon reticulatum* is a freshwater microalga that grows rapidly and causes water bloom. It is mainly composed of cellulose and hemicellulose and accumulates starch as a stored carbohydrate. It can be easily hydrolyzed into glucose and mannose and can be used for microbial fermentation. *H. reticulatum* biomass was used for the production of ethanol production using *Saccharomyces cerevisiae* (Kim et al. 2017b). *P. hysterophorous* was investigated for ethanol production by using separate hydrolysis and fermentation method (Tavva et al. 2016). Hydrolysate was first detoxified by using calcium oxide precipitation method and used for the fermentation by *Torulaspora delbrueckii* R3DFM2, *Schizosaccharomyces pombe* R3DOM3, and *S. cerevisiae* R3DIM4 for ethanol production (Tavva et al. 2016). *Lemna aequinoctialis* (Duckweed) is a fast growing aquatic plant having a growth cycle of 2–3 days and used for the bioremediation of wastewater due to its nutrient adsorbing capacity. Yu et al. used wastewater for the cultivation of duckweed and collected biomass was hydrolyzed using enzymatic methods and subjected for ethanol production using angel yeast (Yu et al. 2014). *Wolffia globose* biomass was explored by Soda et al. for succinate production (200 g Kg^{-1}) using *Actinobacillus succinogenes* ATCC55618 in a simultaneous saccharification and fermentation process (Soda et al. 2015). Weed biomass can also be used to produce higher alcohols. Pretreated *Landoltia punctate* using acidic pretreatment method had been used a starting substrate for fermentation to produce butanol using *Clostridium acetobutylicum* (Su et al. 2014). To make the enzyme productions more cost-effectiveness, different researchers explored weed biomass as a raw material. Saini et al. performed solid-state fermentation of *P. hysterophorus* biomass using *Trichoderma reesei* and able to achieve highest cellulase production in the eighth day of cultivation (Saini et al. 2017). Weed biomass has also been employed for the production of bioplastic such as polyhydroxyalkanoates (PHA). For example, acidic pretreated *P. hysterophorus* biomass was used as a carbon source for *Bacillus aerophilus* fermentation and the process yielded 5.4 g/L of PHA (Chandrika et al. 2017). Some of the efforts to produce various biochemicals such as alcohol and organic acid as well as some bio-macromolecules such as enzymes and polymers have been summarized in Table 8.4.

8.4 Conclusions

Weed plants are conventionally thought to be unwanted and a huge amount of effort is being used to their management. Rapid globalization and increasing population require greener, cheaper, and sustainable source of energy, materials, and chemicals. Science and technology have advanced over the year, so it is the high time to seek the new and innovative utilization of weed plants. Besides using weed biomass

Table 8.4 Various biochemicals produced from weed biomass using microbial fermentation

Weed	Pretreatment	Microorganism	Product	Productivity	Reference
Hydrodictyonreticulatum	Acid hydrolysis	*Saccharomyces cerevisiae*	Ethanol	11.0 g/100 g	Kim et al. (2017b)
	Enzymatic hydrolysis	*Saccharomyces cerevisiae*	Ethanol	22.3 g/100 g	
Parthenium hysterophorus	Chemical and enzymatic	*Torulaspora delbrueckii* R3DFM2	Ethanol	24 g/100 g	Tavva et al. (2016)
		Schizosaccharomyces pombe R3DOM3	Ethanol	27 g/100 g	
		Saccharomyces cerevisiae R3DIM4	Ethanol	27 g/100 g	
Lantana camara	Acidic and enzymatic	*Saccharomyces cerevisiae*	Ethanol	43 g/100 g	Pasha et al. (2007)
Lemna aequinoctialis	Enzymatic treatment	*Saccharomyces cerevisiae*	Ethanol	17 g/100 g	Yu et al. (2014)
Landoltia punctata	Acidic	*Clostridium acetobutylicum*	Butanol	11 g/L	Su et al. (2014)
Ipomoea carnea	Drying	Activity of cellulolytic and acidogenic microbes	Volatile fatty acids (acetic acid, propionic, and butyric acids)	–	Kumar et al. (2015b)
Wolffia globosa	Autoclave and enzymatic	*Actinobacillus succinogenes* ATCC55618	Succinate	20 g/100 g	Soda et al. (2015)
Parthenium hysterophorus	Milling	*Trichoderma reesei*	Cellulase	–	Saini et al. (2017)
Parthenium hysterophorous	Organosolvent	*Talaromycesstipitatus* MTCC 12687	Cellulase and xylanase	–	Bharti et al. (2018)
Eichhornia crassipes	Physical	*Aspergillus niger*	Cellulase	–	Amriani et al. (2016)
Parthenium hysterophorous	Acidic	*Bacillus aerophilus*	PHA	5.4 g/L	Chandrika et al. (2017)
Vetiveria zizanioides	Enzymatic	*Lactobacillus brevis and L. casei*	Xylooligosaccharides	–	Patipong et al. (2019)
Parthenium hysterophorus	Fermentation	*Penicillium oxalicum*	Xylanase	–	Dwivedi et al. (2009)

as a cheaper source of lignocellulose, the weeds can be explored for the important secondary metabolites and bioactive compounds. The huge sources of seaweeds can be explored further for food and nutraceutical application after value additions. Research and development should focus on the screening and identifications of bioactives from the weed plants and their utilization for developing products for agriculture, food, pharmaceuticals, cosmetics, and other societal relevance. However, there are concerns regarding the yields of compounds from the weed plants but considering the cost-effectiveness of initial raw materials, i.e., weed plants and end applications of the bioactive molecules, this is not a deficit deal. Many seaweeds are nutritious and are being used traditionally. So, exploring their nutraceutical potentials, value additions of weed plants are of considerable importance for future food security. Weed plants and alien weeds have numerous inherent properties by which they are growing anywhere without any care. So, a scientific strategy to harness the value out of it is the best way management. This would save a lot of money and effort which are otherwise used for weed management, and on the other hand, it would generate additional revenue in terms of bioproducts. Furthermore, the utilization of weed plants for bioactive compound production can also relieve the burden from medicinal plants and dependency on chemical processes to some extent. In short, the utilization of weed plants for biochemical and bioactive compounds is a strategy from waste to wealth.

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Chapter 9
Utilization of Invasive Weed Biomass for Biochar Production and Its Application in Agriculture and Environmental Clean-up

Ranjit G. Gurav, Shashi Kant Bhatia, Umesh B. Jagtap, Yung-Hun Yang, Yong-Keun Choi, Jingchun Tang, and Amit Bhatnagar

9.1 Introduction

In modern agriculture practices, weeds by advantage of their vibrant and resilient nature pose a constant problem in the agricultural sector and deemed a threat to biodiversity. The common issues linked with invasive species including risk to the native species, excess use of herbicides affects the biological pollinators, competition for the light, water and nutrients that can make a community more vulnerable to re-invasion of weed species (Lindenmayer et al. 2015). Huge biomass is generated through weed eradication processes which can be used as the feedstock to produce biochar through pyrolysis and gasification process. During pyrolysis, cellulosic carbons in the biomass get transformed into the more stable aromatic carbons known as biochar that can be further tailored according to the users' needs (Gurav et al. 2019). Low production cost, high adsorption potential and sustainable properties have captivated the wide application of biochar. Biochar has been widely

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explored for the removal of several contaminants released from industrial, agricultural and residential activities that often pollute the soil, air and water bodies causing the greatest concern (Ahmed et al. 2015; Gurav et al. 2019; Vyavahare et al. 2019). Similarly, biochar addition into the soil has been used as an appropriate and easy access method in developing countries to overcome the above-mentioned problems through increasing soil fertility by decreasing nutrient leaching, increasing soil water-holding capacity and decreasing plant toxicity of pesticide because of biochar's higher specific surface area, higher carbon content and macro- and micro-nutrients (Safaei Khorram et al. 2018).

9.2 Concept of Biochar

Biochar is a solid black carbon obtained after pyrolysis of crop residues, weed biomass, forestry waste, animal manure, food processing waste, paper mill waste, municipal solid waste and sewage sludge at temperatures between 300 to 700 °C under the oxygen-limited conditions (Choi et al. 2020; Lyu et al. 2016; Oliveira et al. 2017). Pyrolysis of biomass typically generates three by-products as biochar (solid), bio-oil (liquid) and gas (syngas), where the composition and yield of by-products vary greatly with the pyrolysis conditions and feedstock (Fig. 9.1) (Gul et al. 2015). Biochar is the low-cost carbonaceous adsorbent with the production cost ranging $0.2–0.5/kg as compared to the ion exchange resins that cost up to $150/kg (Ahmed et al. 2015). The pristine biochar retains several exceptional properties which make it a cost-effective, eco-friendly and efficient asset for its application in the removal of contaminants, carbon sequestration and soil amelioration. However, several research groups are focusing on tailoring the biochar to fascinate its wide applications in wastewater treatment, soil remediation and enhancing the soil properties (Gurav et al. 2019). The physicochemical properties of biochar greatly differ with the feedstock types and pyrolysis conditions that alter the surface area, pH, elemental structure, polarity and atomic ratio leading to variability in adsorption mechanisms (Gurav et al. 2019; Oliveira et al. 2017). Therefore, biochar is considered as the economical alternative over activated carbon for the removal of different organic pollutants like polycyclic aromatic hydrocarbons, antibiotics, pesticides, polychlorinated biphenyls, volatile organic compounds, textile dyes and other inorganic contaminants including heavy metals, phosphate, nitrate, sulphide, etc. (Choi et al. 2020; Choi and Kan 2019; Eun Kim et al. 2020; Gurav et al. 2019; Lyu et al. 2016; Vyavahare et al. 2019; Vyavahare et al. 2018; Zhang et al. 2016b). Besides, when applied to the soil, biochar enhances the water-holding ability, oxygen and moisture content, immobilizes the rhizospheric heavy metals and agrochemicals, carbon sequestration and microbial abundance, with additional benefits of mitigation of climate change (Gul et al. 2015; Gurav et al. 2019; Lyu et al. 2016; Oliveira et al. 2017; Vyavahare et al. 2019; Vyavahare et al. 2018; Zhang et al. 2016b).

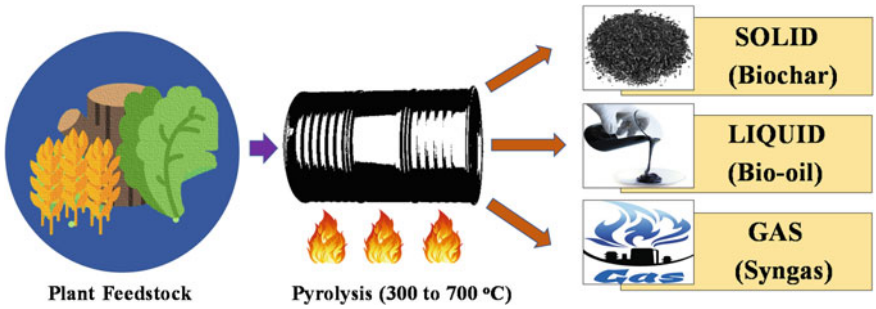

Fig. 9.1 Scheme for biochar production from weed biomass

9.3 Characterization of Biochar

Characterization of the biochar is essential prior to its application for soil and water treatment. Several methods were reported to determine the physicochemical properties and quality of original biomass and its subsequent biochar (Fig. 9.2). Analysis of water leachable petroleum aromatic hydrocarbons (PAHs) that are usually generated due to the incomplete combustion of waste biomass during pyrolysis and heavy metals that could be accumulated in the plants on their exposure to contaminated water or land. These chemicals are listed as the priority pollutants by United State Environmental Protection Agency (US EPA); therefore, a careful examination of water leachable fractions is needed for the biochar before its actual field application (Oleszczuk et al. 2014). In chemical analysis of biochar, pH of the biochar plays an important role in studying pH-dependent phenomena of biochar in agricultural, environmental and biological sciences. Usually, the pH of the biochar is alkaline due to an increase in the alkaline groups, alkali salts like K, Na, Ca and Mg and elimination of acidic functional groups at the higher pyrolysis temperatures (Gurav et al. 2019), whereas electrical conductivity (EC) of biochar is related to the water-soluble ions in biochar which can be determined using biochar-deionized water solution. The exchangeable cations in biochar depend on the feedstock used for biochar preparation, where macro and micro elements in biochar can serve as nutrients in agricultural applications. In the physical characterization of the biochar, investigating the surface area of the adsorbent is essential as it has positive correlations with pollutant removal from soil and aqueous solutions. Brunauer-Emmett-Teller (BET) method is used to calculate the surface area of biochar, where raise in the pyrolysis temperature and resident time increases the surface area, pore volume and average pore size of the biochar (Choi et al. 2020; Gurav et al. 2019; Igalavithana et al. 2017; Lyu et al. 2016). The structural analysis of the biochar and biomass using thermal gravimetric analysis (TGA) is performed to identify the temperature-induced weight loss patterns in material and structural stability at the required heating rate under control atmospheric conditions. Likewise, X-ray diffraction (XRD) is performed for composition and structure

analysis of the pristine, tailored biochar and biomass for the presence and loss of organic compounds such as lignin, cellulose, hemicelluloses and inorganic compounds (Eun Kim et al. 2020; Igalavithana et al. 2017). Furthermore, the surface morphology analysis of biochar is usually performed using scanning electron microscopy (SEM) to evaluate the surface structure and mesopore-micropore distributions, whereas SEM attached with energy dispersive X-ray spectroscopy (EDX) is used to analyze the surface elemental composition of the biochar (Igalavithana et al. 2017). The X-ray photoelectron spectroscopy (XPS) is applied to analyze the surface elements, presence of chemical compounds and chemical bonds on the biochar surface. Likewise, Fourier transform infrared (FTIR) spectroscopy is the non-destructive method used to reveal a degree of carbonization of biochar, functional groups determination and characterization of the biomass feedstock. The proximate analysis of the biochar is basically performed to find the moisture, ash content, volatile matter, fixed C/resident matter in biochar using basic characterization methods. In addition, the ultimate analysis of the biochar primarily focuses on individual elements analysis mainly C, H, N, O and S. Several biochars have a low content of S and N; therefore, C, H and O are the major elements considered in the ultimate analysis. On increasing pyrolysis temperature, H and O contents of biochar decrease; therefore, the H/C and O/C ratios decrease, with enhancing the aromaticity and carbonization (Bhatia et al. 2020; Igalavithana et al. 2017).

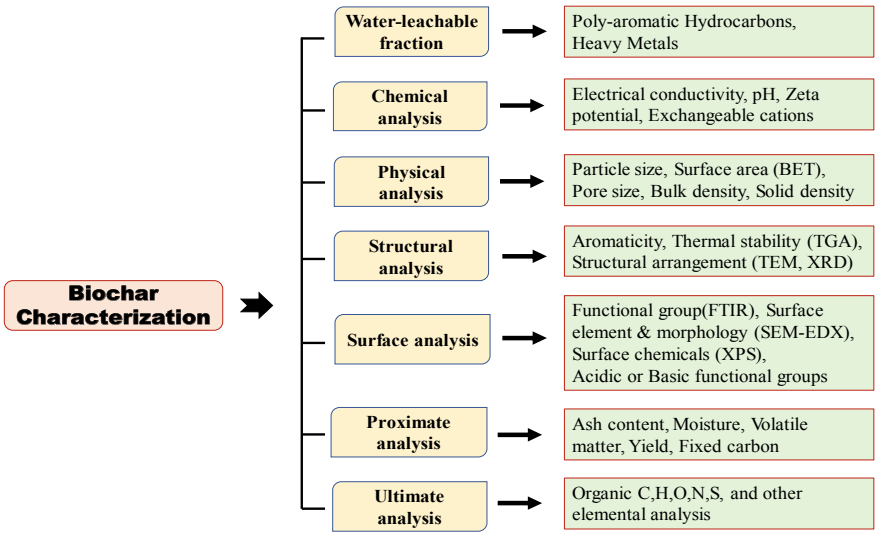

Fig. 9.2 Methods to characterize the biochar

9.4 Invasive Weeds as Feedstock for Biochar Production

Alligator weed (*Alternanthera philoxeroides*), water hyacinth (*Eichhorniacrassipes*), Crofton weed (*Eupatorium adenophorum* Spreng), *Parthenium hysterophorus*, buffalo weed (*Ambrosia trifida* L. var. *trifida)* are the most common invasive weeds found in different countries, where the biomass generated through weeding processes that were used as a feedstock for the biochar production. Morphologies of these weed plants used in biochar preparation have been depicted in Fig. 9.3.

9.4.1 Alligator Weed

Alligator weed (*A. philoxeroides*) is an invasive aquatic weed that originated from South America and has invaded several countries throughout the world. Alligator weed poses a problem by blocking the drainage, and irrigation channels, clogging the turbines in hydro energy plants, reduce the fish population and create water pollution on weed decomposition with an increase in the mosquito breeding areas (Bhattacharjee and Biswas 2018). The disposal of alligator weed through natural decomposition could create a secondary environmental concern by providing feed to pathogens and methane (Huang et al. 2016).

9.4.2 Water Hyacinth Weed

Water hyacinth (*E. crassipes)* is one of the most recalcitrant weeds that originated from the Amazon basin and has been listed among the top 10 worst weeds by the

Fig. 9.3 Weed biomass: **a** Alligator weed; **b** Water hyacinth; **c** Crofton weed; d *Parthenium hysterophorus;* **e** Buffalo weed

International Union for Conservation of Nature (IUCN) (Gopal et al. 2019). This weed creates a serious threat to the ecosystem due to the high reproductive rate, blocking irrigation systems, greenhouse gas emissions from water bodies and damage to the native biodiversity (Masto et al. 2013). At present, physical and mechanical removal of the water hyacinth are the only possible ways to eliminate the weeds from water bodies.

9.4.3 Crofton Weed

Crofton weed (*E. adenophorum* Spreng) is a perennial shrub with 0.8–2.5 m height belonging to family Asteraceae and a top invasive plant in South-West China that has the ability to produce up to 3000 seeds per plant and 40,000 seeds per m^2 in the wild every year (Fan et al. 2019; Li et al. 2014). Due to its high viability, competitiveness and adaptability, the Crofton weed can create severe damage to the vegetation community structure and can affect the ecological environment, agricultural production and landscape. Certain allelochemicals produced by Crofton weed, like dibutyl phthalate, 9-β-hydroxy-ageraphorone and bis(2-ethylhexyl) phthalate can inhibit the plant growth and induce senility in crops (Li et al. 2014). Several methods including chemical, biological and mechanical control were implemented to control Crofton weed.

9.4.4 Parthenium Hysterophorus

*P. hysterophorus*is enlisted in a global invasive species database and considered as the most dangerous weed causing huge loss to biodiversity, economy and health of livestock and human. It is a common invasive species in India, Australia and Africa, and during last century, it has invaded almost all parts of the world (Saini et al. 2014). Its common names are Santa-Maria, white top weed, famine weed, carrot grass and congress grass, etc. This plant produces an allelopathic chemical that suppresses crop yield and acts as an allergen. Its pollen grains also create health problems such as asthma, hay fever, bronchitis, diarrhoea. Generally, *Parthenium* is controlled by using herbicides (paraquat and glyphosate) or weeded by hand, but all these methods are costly. Although *Parthenium* is a weed but many benefits have associated with it, as it has pharmacological and medicinal effects and many other industrial applications. It has been explored for heavy metal removal, enzyme production, biogas and manure production.

9.4.5 *Buffalo Weed*

Buffalo weed (*A. trifida* L.) is an invasive plant originated from North America and has spread in many countries causing a serious loss in the crop yield and creates certain allergic reactions in humans (Ahmad et al. 2014). This plant widely grows along the roadsides and in cultivated crops, where it competes with the native plants. Similarly, the pollens of the weed are significant allergens to humans.

Although different strategic efforts were made earlier to eradicate these noxious weeds, nevertheless, utilization of weed biomass in a sustainable manner was needed. A huge amount of weed biomass is generated globally that could be used as feedstock for the biochar production that can possibly serve the dual purpose of weed biomass utilization and by-products generation for the sustainable management of the weed.

9.5 Production and Applications of Weed Biochar

Weeds are the invasive plants that are noxious by nature and compete with the native plants and agriculture crops. High quantity of weed biomass is produced every year which needs appropriate exploitation technology. One possible way to exploit this biomass is through pyrolysis of biomass to produce biochar which has several applications in agriculture and environmental clean-up. Table 9.1 depicts the preparation and modification of different weed biochar for the removal of environmental contaminants with maximum adsorption capacity and their adsorption mechanism.

9.5.1 *Alligator Weed Biochar*

Alligator weed biochar has been potentially effective for the removal of heavy metals from the soil and water bodies that cause a serious threat to the organisms even at very low concentrations. Yang et al. (2014) tested alligator weed biochar prepared at 600 °C for the adsorption of Pb(II). The experimental data demonstrated a maximum adsorption capacity of 257.12 mg g^{-1} within 2.5 h, which was 5.3 times higher than the activated carbon. The adsorption mechanism for Pb(II) onto biochar was mainly driven by precipitation and complexation of Pb(II) using free carboxyl/hydroxyl functional groups and mineral carbonates of biochar (Yang et al. 2014), whereas Jing et al. (2019) utilized bentonite modified alligator weed biochar (300 °C) for the removal of Cd(II) with a maximum adsorption capacity of 35.3 mg g^{-1} at pH 6 with the involvement of chemisorption mechanism associated with heterogeneous surface and intraparticle diffusion, whereas the thermodynamic studies revealed physisorption which was endothermic and spontaneous (Jing et al.

Table 9.1 Application of weed biochar for the removal of environmental contaminants

Biomass	Pyrolysis temperature and modification of biochar	Targeted pollutants	Maximum adsorption capacity or % removal	Adsorption mechanisms	Reference
Alligator weed	600 °C	Pb(II)	257.12 mg g^{-1}	Precipitation, Complexation	Yang et al. (2014)
	300 °C; Bentonite modified biochar	Cd(II)	35.3 mg g^{-1}	Chemisorption	Jing et al. (2019)
	450 °C; Hydrogen peroxide modified biochar	Metformin hydrochloride	335. 5 µmol g^{-1}	Chemisorption	Huang et al. (2016)
	–	Rhodamine B dye	286 mg g^{-1}	Chemisorption	Du et al. (2018)
Water hyacinth weed	300 °C	Cd	49.837 mg g^{-1}	Chemisorption, Precipitation	Li et al. (2016)
	500 °C		36.899 mg g^{-1}		
	700 °C		25.826 mg g^{-1}		
	393 °C	Cd^{2+}	20.175 mg g^{-1}	Precipitation, Electrostatic attraction, Ion exchange, Complexation, Physical adsorption	Zhou et al. (2019)
	450 °C	Cd^{2+}	74.99 mg g^{-1}	Complexation, Cation exchange, Cation-π interaction, Precipitation,	Ding et al. (2016)
		Pb^{2+}	128.95 mg g^{-1}		
	350 °C; Nanoscale zero-valent iron (nZVI) modified biochar	Cd(II)	56.62 mg g^{-1}	Electrostatic attraction, Complexation, Reduction, Precipitation	Chen et al. (2019)

(continued)

Table 9.1 (continued)

Biomass	Pyrolysis temperature and modification of biochar	Targeted pollutants	Maximum adsorption capacity or % removal	Adsorption mechanisms	Reference
	200–600 °C; Fe_2O_3 modified biochar	Cu^{+2}	3.53 mg g^{-1}	Chemisorption	Nyamunda et al. (2019)
		Zn^{+2}	9.42 mg g^{-1}		
	250 °C; Fe_3O_4 modified biochar	As(V)	7.4 mg g^{-1}	H-bonding, Ligand exchange	Zhang et al. (2016a, b)
	500 °C	Pb(II)	39.09 mg g^{-1}	Chemisorption	Li et al. (2018a)
		Zn(II)	45.40 mg g^{-1}		
		Cu(II)	48.20 mg g^{-1}		
		Cd(II)	44.04 mg g^{-1}		
Crofton weed	300–600 °C	Pb^{2+}	99.30 ± 0.15%	Precipitation	Fan et al. (2019)
		Cd^{2+}	97.49 ± 0.18%		
	500 °C	Flubendiamide	87%	–	Wang et al. (2018)
	500 °C	Acetochlor	–	–	Li et al. (2018b)
	500 °C	Methyl isothiocyanate	87.62 mg g^{-1}	–	Fang et al. (2017)
P. hysterophorus weed	–	Cr(IV)	24.5 mg $g^{-}1$	Chemisorption, Surface diffusion	Venugopal and Mohanty (2011)
	500 °C; NaOH modified biochar	ibuprofen	99%	Chemisorption	Mondal et al. (2016)
	500 °C; NaOH modified biochar	Ranitidine hydrochloride	99%	Chemisorption	Mondal et al. (2017)

(continued)

Table 9.1 (continued)

Biomass	Pyrolysis temperature and modification of biochar	Targeted pollutants	Maximum adsorption capacity or % removal	Adsorption mechanisms	Reference
Buffalo weed biochar	700 °C	Cd(II)	11.63 mg g^{-1}	Complexation, Ion exchange	Yakkala et al. (2013)
		Pb(II)	333.3 mg g^{-1}		
	700 °C	Trichloroethylene	88.47%	Cooperative adsorption	Ahmad et al. (2014)
	700 °C; Alginate immobilized biochar	Cd(II)	9.73 mg g^{-1}	Ion exchange, Electrostatic interactions	Roh et al. (2015)
		TNT	90.09 mg g^{-1}		
		RDX	28.09 mg g^{-1}		
	300 °C	Pb	94%	Precipitation, Ion exchange	Rajapaksha et al. (2015)
		Cu	70%		

2019). Likewise, the alligator weed biochar was explored for the removal of rhodamine B dye, which is widely used in printing, textile, leather, paper and in paint industries. The maximum adsorption capacity of 286 mg g^{-1} was achieved for the alligator biochar, where the chemisorption was the rate-limiting factor with Langmuir isotherm revealing monolayer adsorption mechanism for the dye (Du et al. 2018).

Liu et al. (2011) reported the recovery of nutrients like nitrogen and phosphorus from biochar along with the bio-oil as the by-product from different wetland plants. The low pyrolysis temperature was appropriate to enrich the nitrogen and phosphorus in biochar, and there covery was made through leaching with a total recovery of 76% and 57% for nitrogen and phosphorus, respectively, (Liu et al. 2011). However, phenolic, oxygenated and nitrogenous compounds were the main categories identified in bio-oil with 42.3% recovery from the alligator weed. Therefore, the pyrolysis of wetland plants for the recovery and enrichment of the nutrients from the wetland plants has great potential in water pollution control.

Although biochar comes with several advantages, further modification of the biochar is usually performed to overcome certain deficits of pristine biochar and to improve the broad application of biochar. Huang et al. (2016) modified the alligator weed biochar (450 °C) using hydrogen peroxide and tested for the removal of pharmaceutical compound metformin hydrochloride from the aqueous phase. The data revealed maximum adsorption of 335.5 μmol g^{-1} with chemisorption as the dominant adsorption mechanism using the heterogeneous surface of the biochar, whereas adsorption thermodynamic analysis revealed the adsorption processes which was spontaneous and endothermic for the metformin (Huang et al. 2016).

9.5.2 Water Hyacinth Biochar

Water hyacinth biochar prepared at 300–700 °C was explored for the Cd removal from aqueous solutions. The maximum Cd adsorption capacity was decreased with the increase in pyrolysis temperature, where 49.837 mg g^{-1}, 36.899 mg g^{-1} and 25.826 mg g^{-1} were the adsorption capacity for the biochar prepared at 300, 500 and 700 °C, respectively, (Li et al. 2016). The pH of the solution affected the adsorption rate significantly with pH 5.0 demonstrating 90% Cd removal. Cd adsorption process was followed by the chemisorption mechanism with the dominance of oxygen-containing functional groups and irregular surfaces through esterification reactions. Moreover, the minerals present on the biochar also supported the Cd absorption through precipitation (Li et al. 2016). Another research group optimized the conditions for the biochar preparation from water hyacinth using response surface methodology and used the produced biochar for adsorption of Cd^{2+}. Based on the statistical analysis, the optimal conditions for the water hyacinth biochar preparation were 393 °C temperature, 2.42 h heating time and 15.56 °C min^{-1} as a heating rate (Zhou et al. 2019). The predicted maximum removal rate for Cd^{2+} adsorption was 85.27% with an adsorption capacity of

21.168 mg g^{-1}, whereas the experimental removal rate was 80.70% with the adsorption capacity of 20.175 mg g^{-1}. The dominant mechanisms involved for Cd^{2+} adsorption on water hyacinth biochar were precipitation and electrostatic adsorption, ion exchange, complexation of functional groups and physical adsorption (Zhou et al. 2019). Similarly, Ding et al. (2016) prepared water hyacinth biochar at 300, 450 and 600 °C pyrolysis temperature and applied for the Cd^{2+} and Pb^{2+} removal. Langmuir isotherm model best suited for hyacinth biochar prepared at 450 °C with the maximum adsorption capacity of 74.99 mg g^{-1} (Cd^{2+}) and 128.95 mg g^{-1} (Pb^{2+}) (Ding et al. 2016). The competitive adsorption mechanisms among Cd^{2+} and Pb^{2+} was also considered, where the adsorption kinetics and isotherms suggested that the maximum adsorption capacity of Pb^{2+} was higher than that of Cd^{2+} which was led by the surface complexation, cation exchange, cation–π interaction and precipitation mechanisms responsible for the heavy metal removal (Ding et al. 2016). Likewise, water hyacinth biochar prepared at 500 °C was used for the removal of Pb(II), Zn(II), Cu(II) and Cd(II). The analytical results suggested that alkyl, carboxyl, phosphate and cyano groups present in the biochar played a role in binding the heavy metals effectively. Langmuir model demonstrated maximum removal capacities of 39.09 mg g^{-1}, 45.40 mg g^{-1}, 48.20 mg g^{-1} and 44.04 mg g^{-1} for the removal ofPb(II), Zn(II), Cu(II) and Cd(II), respectively, (Li et al. 2018a).

Similarly, the water hyacinth biochar (350 °C) was tailored using novel nanoscale zero-valent iron (nZVI) and implemented for the removal of Cd(II) from aqueous solutions (Chen et al. 2019). The specific surface area of the pristine biochar was enhanced on the deposition of nZVI, while retaining the functional groups of the carbonaceous surface. Based on the Langmuir isotherm, the maximum Cd(II) adsorption capacity for nZVI-biochar was 56.62 mg g^{-1}that was 2.2 fold higher than pristine biochar. The prominent adsorption mechanism of nZVI-biochar for Cd(II) adsorption was the synergistic activities of metal-ligand coordination by carbonaceous surface functional groups and due to nZVI responsible for electrostatic adsorption, complex formation, reduction and precipitation (Chen et al. 2019). Likewise, the hyacinth magnetic biochar was employed for the removal of Cu^{+2} and Zn^{+2} from aqueous solution (Nyamunda et al. 2019). The Cu^{+2} and Zn^{+2} adsorption matched to the Langmuir isotherm with the maximum adsorption capacity of 3.53 mg g^{-1} and 9.42 mg g^{-1} for Fe_2O_3-biochar while 2.06 mg g^{-1} and 5.99 mg g^{-1} for pristine biochar, respectively, (Nyamunda et al. 2019). In another study, water hyacinth magnetic biochar was explored for the removal of As(V) from aqueous solution (Zhang et al. 2016a). This magnetic biochar was prepared by pyrolyzing the biomass at 250 °C demonstrated higher As (V) removal capacity reaching 7.4 mg g^{-1} based on the Langmuir-Freundlich model. The ligand exchange between the hydroxylated surface of Fe_3O_4 and As(V) anion as well as H bond was mainly responsible for As(V) adsorption. Besides, the water hyacinth biochar was explored for its application in the agriculture sector for improving the soil water retention property and soil enzymatic activities. Bordoloi et al. (2018) applied the water hyacinth biochar prepared at 300–350 °C to the soil and analyzed the water retention property and corresponding crack intensity

capacity in the soil. Among the tested percentage of biochar (0, 2, 5 and 15%), soil added with 15% biochar demonstrated soil water retention capacity of 48.45 ± 0.59% as compared with bare soil 29.5 ± 0.89%, whereas minimum available water content improved from 9.97% to 21.48% for bare soil and 15% biochar added to soil, respectively, (Bordoloi et al. 2018). The addition of biochar to soil also managed to gradual decrease in the corresponding crack intensity capacity from 7 to 2.8%. Masto et al. (2013) showed that the water hyacinth biochar (300–500 °C) has been proved to enhance the microbial biochemical activities in the soil and seedling growth. On supplementing water hyacinth biochar at the concentration of 20 gkg^{-1}, the enzymatic activities in the soil were enhanced for the enzymes like acid phosphatase activity (+32%), alkaline phosphatase (+22.8%) and fluorescein hydrolases (50%) that could be due to increase in the soil biological activities, mainly the active microbial biomass that was enhanced by 3 times and soil respiration by 1.9 times (Masto et al. 2013). Therefore, the utilization of highly problematic invasive weed could provide an alternative way to manage and utilize the weed waste biomass (Zhang et al. 2016a).

9.5.3 Crofton Weed Biochar

Crofton weed is the cheap and locally available weed biomass that can be used for the biochar preparation and further employed as a feasible adsorbent for contaminant removal. Fan et al. (2019) produced Crofton weed biochar at 300, 400, 500 and 600 °C pyrolysis temperature and utilized it for the removal of Pb^{2+} and Cd^{2+} from aqueous solution. The yield of the biochar and H, N and O content was decreased on increasing the pyrolysis temperature, whereas the pH, ash and C, Ca, Mg, P and K content were increased. A solution containing 50 mg L^{-1} of Pb^{2+} or Cd^{2+} was used for the adsorption experiment showed 99.39 ± 0.15% and 97.49 ± 0.18% removal of Pb^{2+} and Cd^{2+}, respectively, (Fan et al. 2019). The mechanism involved in the Pb^{2+} and Cd^{2+} removal was dependent on the amounts of P, Ca, Mg and K that could have helped in precipitation of the heavy metal on biochar surface. Similarly, Meng et al. (2015) used Crofton weed biomass for biochar and syngas production, where the main gaseous products generated in pyrolysis were CH_4, CO_2, CO and H_2. The CH_4 and CO_2 production were decreased on increasing the pyrolysis temperature, while CO and H_2 content were increased.

Crofton weed biochar was also explored in agriculture for the removal of agrochemicals that were applied to control insect pest. Wang et al. 2018 reported the Crofton biochar prepared at 500 °C for the removal of flubendiamide, which is the novel class of insecticide belonging to the class of phthalic acid diamides, and the adsorption capability of Crofton biochar was compared with the synthetic polyoxymethylene material (Wang et al. 2018). Crofton biochar performed well at acidic pH, where the soil sediment having low organic matter content was amended with 5% biochar demonstrated 87% decrease in the flubendiamide content in the

soil. Similarly, Li et al. (2018b) reported supplement of the Crofton weed biochar (500 °C) increased the persistence of the herbicide acetochlor in soil, and further, ageing of the biochar in the soil for long period could increase the bioaccumulation of acetochlor in maize plants. In addition, Crofton weed biochar when applied to the soil delayed the degradation of soil fumigants like methyl isothiocyanate and dimethyl disulphide due to the adsorption of these chemicals onto biochar that eventually decreases the efficiency of these chemicals to control the soil-borne disease (Fang et al. 2017; Han et al. 2017).

9.5.4 Parthenium Hysterophorus *Biochar*

Parthenium biochar produced by pyrolyzing feedstock at 400 °C for 2 h and addition of 4.5 and 9.0 g kg^{-1} of biochar to the soil resulted in increased K content and pH, respectively, which leads to improved microbial activity and soil fertility (Singh et al. 2019). Kumar et al. (2013) also explored *Parthenium* biochar for potential soil applications and found that the addition of the biochar to the soil increased the microbial biomass by 1.4–2.1 times, activities of various enzymes (dehydrogenase and catalase) and seedling vigour index of *Zea mays*(Kumar et al. 2013). Similarly, the industrial wastewater containing toxic metals is a main environmental concern of this century. Venugopal and Mohanty explored biochar from *Parthenium* for the removal of Cr(IV) and reported 24.5 mg g^{-1} adsorption capacity at pH 1.0, temperature 20 °C and reaction time of 160 min. Kinetic analysis results showed that Cr(IV) biosorption followed pseudo second-order kinetics, and surface diffusion was found to be the rate controlling step (Venugopal and Mohanty 2011). Mondal et al. (2016) prepared *Parthenium* biochar (500 °C) by chemical modification (NaOH) method and used it for the removal of non-steroidal and anti-inflammatory drug ibuprofen from contaminated water. Modified biochar showed removal percentage more than 99% at pH 2, temperature 20 °C and incubation time 120 min and followed pseudo second-order kinetics. A similar approach was used by Mondal et al. (2017) for the removal of ranitidine hydrochloride and optimization performed using artificial neural network. *Parthenium* biochar showed 99% removal of ranitidine hydrochloride at pH 2, temperature 20 °C and time 90 min, and adsorption followed pseudo second-order kinetics. Besides, *Parthenium* biomass was prepared by pretreating biomass with sulphuric acid and phosphoric acid, and this pretreated biomass was explored for methylene blue removal in a batch reactor. The adsorption capacity of two adsorbent, i.e. sulphuric acid and phosphoric acid treated were 26.4 mg g^{-1} and 41.6 mg g^{-1}, respectively, and follow both Langmuir and Freundlich isotherm models (Lata et al. 2007).

9.5.5 *Buffalo Weed Biochar*

The buffalo weed biochar was produced at 300, 500 and 700 °C (Yakkala et al. 2013). The resulting biochar (700 °C) was utilized for Cd(II) and Pb(II) removal with the maximum absorption capacities of 11.63 mg g^{-1} and 333.3 mg g^{-1} for Cd (II) and Pb(II), respectively. Surface complexation and ion exchange were the principal mechanisms involved in the adsorption of metal onto buffalo weed biochar (Yakkala et al. 2013), whereas Ahmad et al. (2014) prepared buffalo weed biochar at 300 and 700 °C and found that the higher pyrolysis temperature resulted into higher C-enrichment with loss of H and O-containing functional groups. Produced biochar was used for the removal of trichloroethylene (20 mg l^{-1}) which is a chlorinated hydrocarbon that was classified as a priority pollutant by the US EPA. The buffalo weed biochar prepared at 700 °C demonstrated 88.47% adsorption of trichloroethylene with the cooperative sorption mechanism (Ahmad et al. 2014). Roh et al. (2015) generated buffalo weed biochar (700 °C) and incorporated into alginate to form beads. The biochar-alginate beads were used for the removal of Cd(II) and explosive compounds like 2,4,6-trinitrotoluene(TNT) and 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX). The adsorption mechanism was dominated by ion exchange, and electrostatic interactions with the maximum adsorption capacities of biochar-alginate beads calculated based on the Langmuir equation were 9.73 mg g^{-1}, 90.09 mg g^{-1} and 28.09 mg g^{-1} for Cd(II), TNT and RDX, respectively, (Roh et al. 2015). Besides, different adsorbents including buffalo weed derived biochar (300 and 700 °C), natural iron oxides, gibbsite and silver nanoparticles were used for the immobilization of toxic metal Pb, Cu and Sb from the military shooting range soils (Rajapaksha et al. 2015). The highest immobilization of Pb and Cu was 94% and 70%, respectively, for the biochar prepared at 300 °C with precipitation and ion exchange as the possible adsorption mechanisms (Rajapaksha et al. 2015).

9.6 Conclusion

Weeds are the invasive plants that are noxious by nature and compete with the agriculture crops for nutrients, water and light. Huge weed biomass is generated through the weeding process that has been used as a cost-effective feedstock for biochar production. Biochar is a cost-effective asset that can be applied for the removal of toxic heavy metals, pesticides, pharmaceutical compounds, textile dyes, nutrients, etc. from the soil and water bodies. Tailoring of the pristine biochar for surface modification has broadened the application of the biochar in agriculture and environmental clean-up with more focused targets. Therefore, the utilization of weed biomass for biochar production serves the dual purpose of weed biomass management and biochar production that can be used for value addition for the sustainable management of the weed.

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Chapter 10
Weed Biomass-Based Nanoparticles and Their Applications

Umesh B. Jagtap and Ranjit G. Gurav

10.1 Introduction

To remain completely acquainted with a book chapter, one must know the word weed. There have been numerous definitions of weeds. In Merriam-Webster dictionary, a weed is defined as '*a plant that is not valued, where it is growing and is usually of vigorous growth; especially: one that tends to overgrow or chokes out more desirable plants*'. The weed society defines a weed is '*any plant or vegetation, excluding fungi, interfering with the objectives or requirements of people*' (EWRS 1986; Zimdahl 2018) or 'a plant growing, where it is not desired' (Buchholtz 1967; Zimdahl 2018). This leads to understanding that the weeds are uncultivated, undesirable plants associated with our crop plants and somehow hinders or impedes with human activities. The weeds are responsible for various agricultural, environmental and economic losses. They not only interfere with agricultural activities like crop harvesting but also accountable for economic losses by reducing crop health, quality and yield. Weeds are also interfering with animal feeding by causing poisoning, tainting animal products (e.g. undesirable flavour to milk) and act as an alternate/intermediate host for plant and animal pests and parasites. Besides this, they prevent the water flow by blocking the ditches and irrigation channels. Weeds are harmful and often must be controlled (Naylor and Lutman 2002). Generally, weeds are difficult to eradicate and control due to the number of characteristics as shown in Fig. 10.1 (Baker 1965; Naylor and Lutman 2002).

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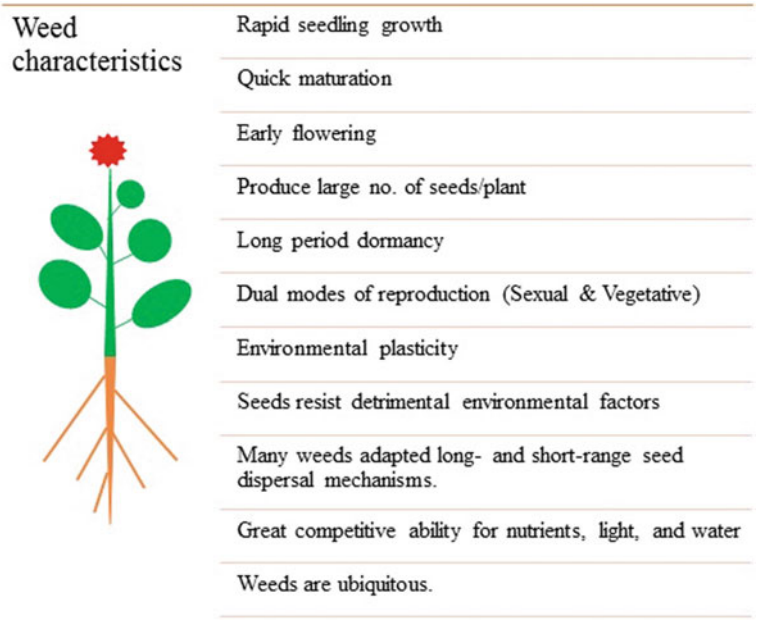

Fig. 10.1 Characteristics of weeds

Nowadays, the weeds and their management are a challenging and key problem in agriculture, the environment and society. The weed control carried out by several mechanical, biological and chemical methods. Each method has its own advantages and disadvantages. The widespread use of herbicides causes environmental pollution and many problems. Hence, there is a need to explore alternative strategies to control invasive weeds. Nanoparticles are the major building blocks in nanotechnology. The unique optoelectronic and physiochemical properties of NPs also open numerous possibilities in biological, chemical, material, physical and engineering research (Huang et al. 2011; Njagi et al. 2011). The different chemical and physical methods were commonly used for the synthesis of nanoparticles. The chemical methods involve the use of organic solvents, toxic reducing and stabilizing agents causing environmental pollution. Furthermore, non-biodegradable toxic stabilizing agents may also limit their use in medical fields (Narayanan and Sakthivel 2011). In contrast, biological methods not only inexpensive but also makes synthesis of nanoparticles eco-friendlier decreasing the use of chemicals and producing less waste. The biological methods utilize bacteria, fungi, algae and plants to facilitate nanoparticle synthesis through the green chemistry approach (Mulvihill et al. 2011; Virkutyte and Varma 2011).

Recently, phytosynthesis approach utilizing weed plants/weed extract has opened up new avenues for inexpensive, simple, rapid, stable and environment friendly synthesis of nanoparticles. The advantage of phytonanotechnology includes biocompatible, scalable and clinically applicable nanoparticle synthesis in

an aqueous medium (Noruzi 2015; Singh et al. 2016). Therefore, weed-based biosynthesis of nanoparticles is considered as an alternative strategy not only for the utilization of the invasive weed species but also for weed control through its harvesting and usage.

10.2 Nanoparticles Synthesis Using Weed and Weed Extract

Over the past few years, various weed plants studied and employed for the phytosynthesis of metal nanoparticles (Table 10.1). For the phytosynthesis of nanoparticles by weed plant extracts, the entire plant/different weed plant parts (root, leaf, stem, flower, fruit, etc.) are washed thoroughly with distiled water to remove the associated microorganisms and dirt. The samples were blotted to dry and then cut into small pieces. Then, sample was boiled in water to obtain the extract for nanoparticle synthesis. Afterwards, the extract can be passed through nylon mesh and purified by filtration through Whatmann filter paper and centrifugation. The varying concentrations of plant extract and metal salt solution in different volume ratios are mixed together, and the reaction mixture was incubated at room temperature for nanoparticle synthesis. The change in colour (e.g. brown for AgNPs and red or purple for AuNPs) indicates the synthesis of nanoparticles. After completion of incubation time centrifuge the reaction mixture to pellet the NPs and pour off the supernatant. Then wash the NPs by re-suspending the pellet in water or solvent to remove impurities. Lastly, after centrifugation, NPs collected in the form of a bottom pellet (Singh et al. 2016). Further, the biosynthesized nanoparticles can be characterized by using different analytical techniques (Fig. 10.2).

10.3 Characterization of Nanoparticles

Recent advancement and availability of sophisticated physical methods and instruments lead to rapid progress in nanoscience and nanotechnology. Nanoparticles generally characterized based upon their sizes, shapes, morphology, structure, surface area, microstructure, atom-level structure and electronic structure. For an overview of the characterization techniques used for nanoparticle characterization, see the following articles and references therein (Rao and Biswas 2009; Patra and Baek 2014). The common techniques involved in the characterization of nanoparticles are given in Table 10.2.

Table 10.1 Biosynthesis of nanoparticles using weeds

Name of weed	Plant part	Type of NPs	Time	Instruments	Shape	Size (nm)	Application	Reference
Antigonon leptopus	Leaf, stem, root	Gold	1–120 h	UV-VIS, SEM, HR-SEM, TEM, SAED, XRD, DLS, FTIR	Isotropic spherical and anisotropic triangular, pentagonal, hexagonal and irregular	25–45	Degradation of textile dyes	Ganaie et al. (2016c)
Chenopodium murale	Leaf	Silver	24 h	UV-VIS,TEM	_	30–50	Antioxidant and antibacterial activity	Abdel-Aziz et al. (2014)
Calotropis procera	Flower	Silver	2–16 h	UV-VIS, SEM-EDX, XRD, FTIR	_	35	_	Babu and Prabu (2011)
Cannabis sativa	Stem	Gold and silver	2.5 and 8 min	UV-VIS, TEM-SAED, AFM, DLS, FTIR, sp-ICP-MS	Spherical	20–40	Inhibition of biofilms	Singh et al. (2018)
Chenopodium aristatum L	Stem	Silver	6 h–8 d	UV-VIS, TEM, XRD, ICP-MS, FTIR,	Quasi-spherical	3–36	Degradation of 4-nitrophenol, antibacterial activity	Yuan et al. (2017)
Clitoria ternatea	Leaf	Silver	60 min	UV-VIS, FTIR, XRD, SEM	Spherical	*ca.* 20	Antibacterial activity	Krithiga et al. (2015)
Solanum nigrum	Leaf	Silver	60 min	UV-VIS, FTIR, XRD, SEM	Spherical	*ca.* 28	Antibacterial activity	Krithiga et al. (2015)
Cynodon dactylon	Leaf	Silver	5–60 min	UV-VIS, XRD, SEM, TEM	Spherical	8–10	Antibacterial activity	Sahu et al. (2013)
Desmodium triflorum	Whole plant	Silver	3 h	UV-VIS, TEM, XRD	Spherical	5–20	Antibacterial activity	Ahmad et al. (2011)
Euphorbia hirta	Leaf	Silver	10 min	UV-VIS, SEM	Spherical	40–50	Antibacterial activity	Elumalai et al. (2010)
Mimosa pudica	Leaf	Silver	2 h	UV-VIS, SEM, TEM, XRD, DLS	Spherical	10–60	_	Ganaie et al. (2015)

(continued)

Table 10.1 (continued)

Name of weed	Plant part	Type of NPs	Time	Instruments	Shape	Size (nm)	Application	Reference
Ipomoea carnea	Leaf, stem, root	Silver	4 h	UV-VIS, HR-SEM, TEM, XRD, FTIR	Spherical	–	Degradation of organic pollutants	Ganaie et al. (2014)
Lantana camara	Leaf	Silver	30 min	UV-VIS, FESEM, TEM, DLS, XRD	Round and rod needle	~40 ± 2.8	Antibacterial activity	Singh et al. (2015)
Lantana camara	berry	Silver	120 h	UV-VIS, DLS, TEM-SAED	Spherical	~75.2	Antioxidant activity	Kumar et al. (2015)
Lantana camara	Leaf	Silver	5–10 min	UV-VIS, XRD, XPS, SEM-EDS, FESEM, AFM, TEM, FTIR, PHOTOLUMINESCENCE SPECTROSCOPY, ZETA POTENTIAL	Spherical	14–27	Antibacterial activity	Ajitha et al. (2015)
Lepidium draba	Root	Silver	15 min	FESEM, TEM, FTIR,	Spherical	20–80	Antibacterial activity	Benakashani et al. (2017)
Lantana camara L	Leaf	Silver	10 min	UV-VIS, HRSEM-EDAX, TEM-SAED, XRD, FTIR, XPS	Spherical	~ 33.8	Antioxidant and antibacterial activity	Manjamadha and Muthukumar (2016)
Parthenium hysterophorus L	Leaf	Silver	10 min	UV-VIS, TEM	Irregular	~50	_	Parashar et al. (2009)
Parthenium hysterophorus L	Leaf	Zinc oxide	4–5 h	UV-VIS, FTIR, SEM, TEM	Spherical hexagonal	27 ± 5 84 ± 2	Antifungal activity	Rajiv et al. (2013)
Mimosa pudica	Leaf, stem, root	Gold	96–120 h	UV-VIS, SEM, HR-SEM-EDAX, TEM, FTIR	Spherical	1–2	Catalytic and antioxidant activity	Pirathiba et al. (2018)

(continued)

Table 10.1 (continued)

Name of weed	Plant part	Type of NPs	Time	Instruments	Shape	Size (nm)	Application	Reference
Pistia stratiotes	Aerial and submerged plant parts	Gold	6 h	UV-VIS, SEM, HR-SEM-EDAX, TEM, XRD, FTIR	Spherical, triangular, hexagonal, pentagonal and truncated triangular shaped	2–40	_	Anuradha et al. (2015)
Prosopis juliflora	Leaf	Silver	5 min	UV-VIS, XRD, SEM, FTIR	Polygonal	11–19	Antibacterial activity	Raja et al. (2012)
Solidago altissima	Leaf	Silver	60 min	UV-VIS-NIR, SEM-EDS, TEM, XRD, XPS	_	_	Plasmonic photocatalyst	Kumar et al. 2016
Trianthema decandra	Root	Gold	3 h	UV-VIS, FESEM-EDX, FTIR	Spherical, cubical, triangular and hexagonal	33–65	Antibacterial activity	Geethalakshmi and Sarada (2012)
Trianthema decandra	Root	Silver	6–24 h	UV-VIS, FESEM-EDX, FTIR	Spherical	36–74	Antibacterial activity	Geethalakshmi and Sarada (2012)
Tinospora cordifolia	Whole plant	Gold	10 min	UV-VIS, HR-SEM-EDAX, TEM-SAED, XRD, FTIR	Spherical or polydispersed triangular, pentagonal and hexagonal	16–75	_	Abbasi et al. (2014)
Cassia hirsuta	Leaf	Silver	30 min	UV-VIS, SEM-EDX, FESEM, TEM, FTIR, XRD	Spherical	6.9 ± 0.1	Mosquito larvicidal potential against *Culex quinquefasciatus*	Adesuji et al. (2016)
Hypericum perforatum	Leaf	Silver	24 h	UV-VIS, TEM, XRD, DLS,	_	<40	Antibacterial activity	Tortella et al. (2019)
Malva nicaensis	Leaf	Silver	24 h	UV-VIS, TEM, XRD, DLS,	_	<40	Antibacterial activity	Tortella et al. (2019)

(continued)

Table 10.1 (continued)

Name of weed	Plant part	Type of NPs	Time	Instruments	Shape	Size (nm)	Application	Reference
Chenopodium album	Leaf	Silver and gold	15 min–2 h	UV-VIS, TEM, EDX, FTIR	Quasi-spherical	10–30	_	Dwivedi and Gopal (2010)
Eichhornia crassipes	Cellulose solution	Silver	5 min–50 h	UV-VIS, TEM, FTIR	Spherical	2.68–5.69	_	Mochochoko et al. (2013)
Commelina nudiflora L.	Whole plant	Gold	15 min–24 h	UV-VIS, FESEM-EDX, XRD, FTIR	Spherical	50–50	Antioxidant and antibacterial activity	Kuppusamy et al. (2015)
Antigonon leptopus	Leaf, stem, root	Palladium	30 min	UV-VIS, SEM, HR-SEM-EDX, TEM, FTIR, XRD	Spherical	5–70	_	Ganaie et al. (2016a)
Antigonon leptopus	Leaf, stem, root	Platinum	20–40 min	UV-VIS, SEM, HR-SEM-EDX, TEM, FTIR, XRD	Spherical	5–190	_	Ganaie et al. (2018)
Eichhornia crassipes	Leaf	Iron	_	UV-VIS, DLS, FESEM-EDX, TEM, XRD, FTIS	Spherical	40–60	Wastewater remediation	Prabhakar and Samadder (2017)
Lantana camara	Leaf	Iron	_	UV-VIS, DLS, FESEM-EDX, TEM, XRD, FTIS	Irregular	50–80	Wastewater remediation	Prabhakar and Samadder (2017)
Mimosa pudica	Leaf	Iron	_	UV-VIS, DLS, FESEM-EDX, TEM, XRD, FTIS	quasi-spherical	65–230	Wastewater remediation	Prabhakar and Samadder (2017)
Antigonon leptopus	Leaf, stem, root	Bimetallic silver–gold	2 h	UV-VIS, HR-SEM-EDAX, XPS, XRD, FTIR	Spherical	10–60	_	Ganaie et al. (2016b)
Salvinia molesta	Aerial and submerged plant parts	Gold	48 h	UV-VIS, HR-SEM-EDAX, TEM-SAED, XRD, FTIR	Spherical, triangular, pentagonal, and nanoflowers	7–25; 20–50; 75–175	_	Abbasi et al. (2016)

(continued)

Table 10.1 (continued)

Name of weed	Plant part	Type of NPs	Time	Instruments	Shape	Size (nm)	Application	Reference
Eichhornia crassipes	Leaf	Zinc oxide	6 h	UV-VIS, XRD, SEM-EDX, TEM-SEAD	Spherical	32 ± 4	_	Vanathi et al. (2014)
Chromolaena odorata	Leaf	*Magnesium oxide*	12 h	UV-VIS, SEM-EDX, XRD, TEM, FTIR, GC-MS	Cubic	~12.3	_	Essien et al. (2020)
Mimosa pudica	Leaf	Silver	24 h	UV-VIS, SEM-EDAX, FTIR, GC-MS	Spherical	~100	Antibacteria activity, antifungal activity	Gopinath et al. (2020)
Plantago major	Whole plant without leaves	Silver	24 h	UV-VIS, HR-SEM-EDS, TEM, XRD	Spherical	~25	Antibacteria activity, antifungal activity	Küünal et al (2019)
Prosopis juliflora	Leaf	Silver	_	UV-VIS, SEM, XRD, FTIR	Spherical	~30	Antibacterial, photocatalytic and biosorption activity	Malini et al. (2020)

10.4 Factors Responsible for Weed-Based Phytosynthesis of Nanoparticles

The phytosynthesis and stabilization of NPs are affected by several factors, such as plant metal extract concentration ratios, pH of the reaction medium, reaction temperature and incubation time.

10.4.1 Plant Extract and Metal Concentration Ratios

The crude extracts and isolated metabolites obtained from various parts of the weed plants are used for metal NPs synthesis (Table 10.1). The phytochemical constituents and composition are greatly varied amongst the plants and plant parts of the same plant. Therefore, the finding of the optimum plant extract and metal salt solution concentrations in appropriate ratios is the essential step to establish a reproducible protocol for economic and efficient production of nanoparticles with homogenous size, shape and morphology. Ganaie et al. (2016c) studied the effect of various concentrations of extracts obtained from leaves, stems and roots of the *Antigonon leptopus* plant on the gold nanoparticle formation. The metal to plant extract (1:12 and 1:15) ratios resulting in the formation of isotropic and spherical gold nanoparticles. However, higher metal to plant extract ratios results in polydisperse, anisotropic gold nanoparticles. In another study, Singh et al. (2015) reported that amongst the various concentrations of *Lantana camara* leaf extracts and silver nitrate solution tested, 10% leaf extract and 3 mM $AgNO_3$ solution were the most suitable combination for size controlled Ag^0 nanoparticles synthesis. It was confirmed that aqueous extracts prepared from leaves, stem and roots of *Ipomoea carnea* plant gave fairly similar and reproducible formation of silver nanoparticles but differ in the rate of synthesis and characteristics of AgNPs. Therefore, it is possible to control the shape and size of NPs by changing a reaction mixture (metal to plant extract) concentration ratios (Ganaie et al. 2014).

10.4.2 pH of the Reaction Medium

The pH of the reaction medium is a very important factor influencing the synthesis of nanoparticles. Ganaie et al. (2016c) used *A. leptopus* plant part extracts for the synthesis of gold nanoparticles. Authors of the study observed that no gold nanoparticle formation takes place at pH 1 or lower while nanoparticles formation started at pH 2 and improved considerably at pH 3.

Furthermore, the gold nanoparticles formed at pH 2 ($\sim$55 nm), aggregated and precipitated rapidly after formation, while smaller, spherical and stable nanoparticles formed at pH $\geq$ 3. In another report, the more silver nanoparticles were

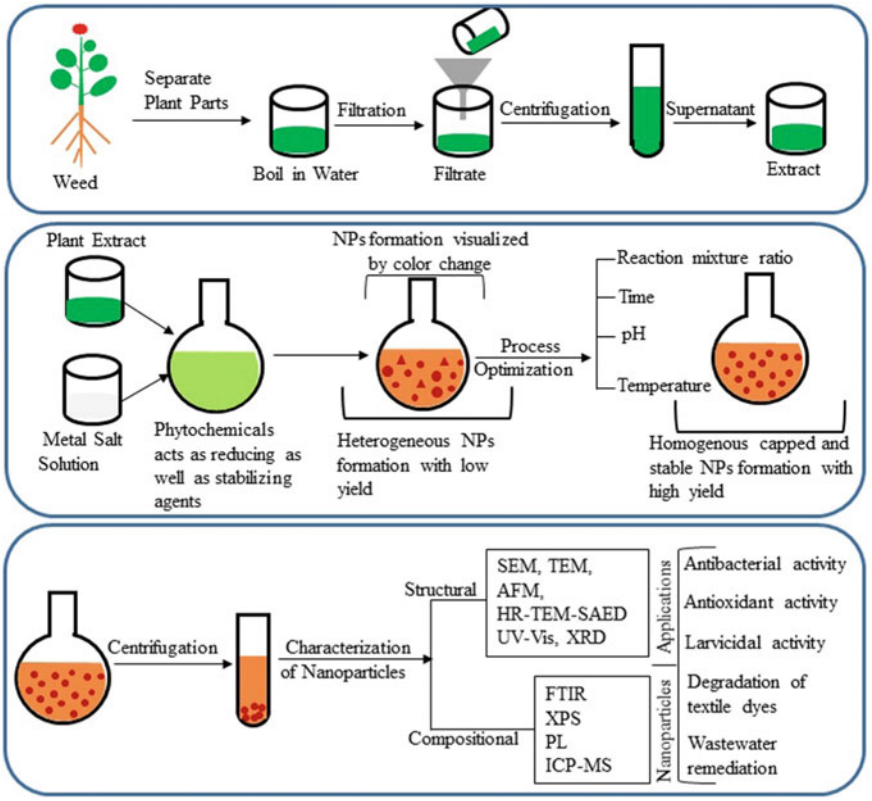

Fig. 10.2 Schematic of the NPs synthesis, characterization and applications. Going from the preparation of extract from weed plant to applications

synthesized using *Chenopodium aristatum* stem extract under higher alkaline conditions (pH = 12), and no significant synthesis was observed under acidic conditions (Yuan et al. 2017). Similarly, phytosynthesis of silver nanoparticles by *Clitoria ternatea* and *Solanum nigrum* leaf extract inhibited in an acidic environment and improved in basic condition. The large nanoparticles were synthesized at pH 4, whereas small and greatly dispersed nanoparticles produced at pH 9 as compared to normal sized nanoparticles formed at neutral pH (Krithiga et al. 2015). The pH can in fact change the charge of natural phytochemicals affecting their binding capacity and reduction potential for metal ions, thus affecting nanoparticles morphology and yield during nanoparticles production (Singh et al. 2016).

Table 10.2 Techniques/methods used for nanoparticle characterization

Type of characterization	Techniques involved	Acronym	Information obtained about
Nanoparticle formation	UV-visible spectroscopy	UV-vis spectroscopy	Size, structure, stabilization and aggregation of NPs
Particle size and morphology	High-resolution transmission electron microscopy	HR-TEM	Size, shape and structural information
	Transmission electron microscopy	TEM	Size, shape and structural information
	Transmission electron microscopy selected area electron diffraction	TEM-SAED	The orientations, atomic arrangements and structures of narrow regions of interest in nanomaterials
	Scanning electron microscopy	SEM	Surface morphology
	Field emission scanning electron microscopy	FESEM	Surface morphology
	Atomic force microscope	AFM	Morphology and electronic states of nanomaterials of different dimensionalities
	Dynamic light scattering	DLS	Particle size distribution
Surface hydrophobicity/ charge	Zeta potential measurement	_	Stability and surface charge of the colloidal nanoparticles
	X-ray photoelectron spectroscopy	XPS	Quantitative information about elemental composition, empirical formula, chemical state and electronic state of the elements within nanomaterials.
	Fourier transform infrared spectroscopy	FTIR	Identification of molecules covering nanomaterial surfaces
	Photoluminescence spectroscopy	PL	Electronic structure of both intrinsic and extrinsic semiconducting nanomaterials
Crystallinity	X-ray diffraction	XRD	Gross crystal structure of nanomaterials
Elemental analysis	Energy dispersive X-ray spectra	EDAX/EDX	Elemental analysis of nanomaterials
Other techniques	Single-particle inductively coupled plasma mass spectrometry	sp-ICP-MS	Size fractionation and quantification of synthesized nanoparticles

10.4.3 Reaction Incubation Time

The incubation period for nanoparticle reaction mixture significantly affects the quality, morphology and stability of the synthesized nanoparticles. The time required for the biosynthesis of different metal nanoparticles by various plant species and extracts is greatly varies (Table 10.1). The surface plasmon resonance (SPR) absorption for nanoparticle formation increases as the incubation time increased (Ahmad et al. 2011; Babu and Prabhu 2011; Krithiga et al. 2015). The SPR signal influenced by the size, shape, and composition of the NPs formed (Babu and Prabhu 2011). The long incubation time results in aggregation of nanoparticles which lead to an increase in the size of the nanoparticles as compared to the short/optimum incubation time (Ganaie et al. 2015; Krithiga et al. 2015). Therefore, optimum reaction incubation time is required for completion of reaction and formation of suitable sized nanoparticles.

10.4.4 Reaction Temperature

The stability, activity and chemical characters of the NPs are influenced by the temperature of the reaction mixture. Also, temperature speeds up the rate of reaction and nanoparticle formation. For instance, the time required for the synthesis of silver nanoparticles by *C. aristatum* stem extract has been reduced from 6 h to 5 min by increasing temperature of the reaction mixture to 90 °C (Yuan et al. 2017). The biosynthesis of gold nanoparticles with *A. leptopus* leaf extract increases with rise in temperature of the reaction mixture to 70 °C as compared to 29 °C. Furthermore, it also leads to the formation of isotropic, monodisperse, stable gold nanoparticles (Ganaie et al. 2015). Similarly, the gold nanoparticles synthesized by using *Cannabis sativa* stem extracts showed less polydispersity when the reaction is carried out at 100 °C but furthermore rise in temperature results in loss of stability and agglomeration of nanoparticles (Singh et al. 2018).

10.5 Applications of Nanoparticles

10.5.1 Antibacterial Activity

Silver has been used as an antibacterial agent since early times. Therefore, the silver nanoparticles have been used as antibacterial agents in several medical devices and food packaging. The antibacterial activity of nanoparticles is listed in Table 10.3. To evaluate the antibacterial activity of NPs, the inhibition zone diameter (IZD) and minimum inhibitory concentration (MIC) taken into consideration. Though AgNPs is a widely used antibacterial agent, the precise mode of action of AgNPs is partly

Table 10.3 Antibacterial activities of nanoparticles synthesized using weeds

Weed plant	NPs	Assay method	Bacterial strain	IZD (mm)	MIC	References
Chenopodium murale	Ag	Cup plate agar diffusion	*Staphylococcus aureus*	–	–	Abdel-Aziz et al. (2014)
Cannabis sativa	Ag		*P. aeruginosa* Pa01	–	6.25 µg/ml	Singh et al. (2018)
			E. coli UTI89	–	12.5 µg/ml	
			S. epidermidis ATCC 35984	–	>50 µg/ml	
Chenopodium aristatum	Ag	Cup plate agar diffusion	*E. coli*	–	–	Yuan et al. 2017)
			Staphylococcus aureus	–	–	
Clitoria ternatea, *Solanum nigrum*	Ag	Disc diffusion	*Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa and Klebsiella aerogenes*	5–20	–	Krithiga et al. 2015
Cynodon dactylon	Ag	Agar well diffusion	*P. aeruginosa*	12	–	Sahu et al. 2013
			S. aureus	12	–	
			E. coli	6	–	
			S. typhymurium	7	–	
Desmodium triflorum	Ag	–	*Staphylococcus spp.*	–	53 µg/ml	Ahmad et al. 2011
			Escherichia coli	–	27	
			Bacillus subtilis	–	54	
Lantana camara	Ag	contact and disc diffusion method	*Escherichia coli* (ATCC 8739)	–	50 ppm	Singh et al. (2015)
			Escherichia coli (ATCC 8739)	–	60 ppm	
Lantana camara	Ag	Disc diffusion	*E. coli*	6	–	Ajitha et al. 2015
			Staphylococcus spp	6	–	
			Pseudomonas spp.	7	–	
			Bacillus spp.	8	–	
Lepidium draba	Ag	Disc diffusion	*S. aureus* (ATCC 29213)	10.8	–	Benakashani et al. 2017
			B. cereus (ATCC 14579)	10.8	–	
			E. coli (ATCC35218)	10.8	–	
			S. typhimurium (ATCC 14028)	10.4	–	

(continued)

Table 10.3 (continued)

Weed plant	NPs	Assay method	Bacterial strain	IZD (mm)	MIC	References
Lantana camara L	Ag	Agar well diffusion	*Staphylococcus aureus*	25	–	Manjamadha and Muthukumar (2016)
			Escherichia coli	28	–	
			Pseudomonas aeruginosa	27	–	
			Klebsiella pneumonia	30	–	
Trianthema decandra	Ag	Disc diffusion method	*Staphylococcus aureus* (MTCC 29213)	13.5	–	Geethalakshmi and Sarada 2012
			Streptococcus faecalis (MTCC0459)	15.5	–	
			Enterococcus faecalis (MTCC 2729)	14	–	
			Escherichia coli (MTCC 443)	15.5	–	
			Pseudomonas aeruginosa (MTCC 1035)	14.5	–	
			Proteus vulgaris (MTCC 1771)	20.5	–	
			Bacillus subtilis (MTCC 121)	12	–	
			Yersinia enterocolitica (MTCC 840)	17.5	–	
	Au		*Staphylococcus aureus* (MTCC 29213)	14.5	–	Geethalakshmi and Sarada 2012
			Streptococcus faecalis (MTCC0459)	13.5	–	
			Enterococcus faecalis (MTCC 2729)	10.5	–	
			Escherichia coli (MTCC 443)	9.5	–	
			Pseudomonas aeruginosa (MTCC 1035)	11.5	–	
			Proteus vulgaris (MTCC 1771)	15	–	
			Bacillus subtilis (MTCC 121)	9.5	–	
			Yersinia enterocolitica (MTCC 840)	15.5	–	

(continued)

Table 10.3 (continued)

Weed plant	NPs	Assay method	Bacterial strain	IZD (mm)	MIC	References
Mimosa pudica	Ag	Well diffusion assay	*Bacillus subtilis*	0.6 ± 0.5	–	Gopinath et al. 2020
			Staphylococcus aureus	1.5 ± 0.0	–	
			Micrococcus luteus	1.53 ± 0.3	–	
			E. coli	1.93 ± 0.3	–	
			Pseudomonas aeruginosa	1.96 ± 0.3	–	
			Proteus vulgaris	2.03 ± 0.3	–	
			Candida strain 183	1.86 ± 0.3	–	
			Candida strain 184	2.36 ± 0.3	–	
Plantago major	Ag		*E. coli*		1.6 µg/ml	Küünal et al. (2019)
			S. aureus		0.8 µg/ml	

known. The several mechanisms such as the release of silver nanoparticles and silver ions, cell membrane damage, DNA interaction, free radical production were responsible for the antibacterial activity of AgNPs. For more details on account of antimicrobial mechanisms of AgNPs and their action on bacteria see the following review articles: Durán et al. (2016); Khalandi et al. (2017); Tang and Zheng (2018).

10.5.2 Antifungal Activity

Rajiv et al. (2013) evaluated antifungal activities of zinc oxide nanoparticles synthesized by *Parthenium hysterophorus* leaf extract against various fungal strains by using Kirby Bauer disc diffusion method in vitro, and amphotricin B (10 µg/ml) used as a positive control. The ZnO nanoparticles (50 µg/ml and 27 ± 5 nm) showed the highest zone of inhibition against *Aspergillus flavus* (MTCC-7589) (24.66 ± 0.57 mm) and lowest against *Fusarium culmorum* (MTCC-2090) (14 ± 0.57 mm) as compared to the positive control (19.66 ± 0.57 mm). In another study, Geethalakshmi and Sarada (2012) assessed the antifungal activity of silver and gold nanoparticles synthesized by *Trianthema decandra* root extract against fungus *Candida albicans*. The silver nanoparticles showed more growth inhibition of *C. albicans* (IZD = 14.5 mm) as compared to the gold nanoparticles (IZD = 8.5 mm).

10.5.3 Antioxidant Activity

The antioxidant activity of the aqueous leaf extract and silver nanoparticles synthesized by leaf extract of *Chenopodium murale* was evaluated using DPPH (2, 2-diphenyl-2-picrylhydrazyl) scavenging and β-carotene bleaching assays. The result shows that the silver nanoparticles possess the higher DPPH scavenging and β-carotene oxidation antioxidant activity compare to the plant extract alone in a dose dependent manner (Abdel-Aziz et al. 2014). Similarly, the silver nanoparticles synthesized by using *L. camara* berry extract showed higher DPPH scavenging activity than the berry extract alone (Kumar et al. 2015). Also, the AgNPs synthesized by *L. camara* leaf extractable to scavenge DPPH free radicals (Manjamadha and Muthukumar 2016). Kuppusamy et al. (2015) studied the antioxidant activity of gold nanoparticles synthesized by *Commelina nudiflora* L. plant with DPPH and ABTS assays. The result revealed that AuNPs were found to scavenge the stable DPPH (IC_{50} = 20 μg/ml) and ABTS (IC_{50} = 40 μg/ml) radical. In another study, *Mimosa pudica* biosynthesized gold nanoparticles showed DPPH scavenging activity (Pirathiba et al. 2018). The possible antioxidant activity of biosynthesized nanoparticles is related to their high surface area to volume ratio and the presence of antioxidant biomoities on their surfaces (Manjamadha and Muthukumar 2016; Pirathiba et al. 2018)

10.5.4 Larvicidal Activity

Mosquitoes are important vectors for the transmission of many dreadful diseases. *Culex quinquefasciatus* mosquito is a vector of lymphatic filariasis commonly known as elephantiasis. The most chemical control methods were employed to kill the larvae, and adult mosquitoes lead to environmental pollution. Therefore, there is a need to develop new environment friendly measures to control mosquitoes. The silver nanoparticles synthesized using *Cassia hirsuta* leaf extract showed larvicidal activity against *C. quinquefasciatus* having LC_{50} = 4.43 ppm and LC_{90} = 8.37 ppm (Adesuji et al. 2016).

10.5.5 Catalytic Activity

The biosynthesized gold nanoparticles were used for the catalytic degradation of textile dyes Congo red and Remazol Brilliant Blue R (Ganaie et al. 2016c). Similarly, silver nanoparticles synthesized using *I. carnea* were play a role in catalyzing degradation of Alizarin Red S and Remazol Brilliant Blue R dyes (Ganaie et al. 2014). In another report, Pirathiba et al. (2018) show that the gold nanoparticles biosynthesized by *M. pudica* plant act as catalyst in the presence of

reducing agents such as sodium borohydride, able to degrade 4-nitrophenol (4-NP) to 4-aminophenol (4-AP). Also, the synthesized AgNPs using *C. aristatum* stem extract exhibited strong catalytic activity in degradation of 4-NP (Yuan et al. 2017). Therefore, gold and silver NPs can be used to catalyze degradation of organic pollutants.

10.5.6 Wastewater Remediation

Prabhakar and Samadder (2017) utilized aquatic (*Eichhornia crassipes*) and terrestrial (*L. camara* and *M. pudica*) weeds for the synthesis of iron nanoparticles. The biosynthesized iron nanoparticles were utilized for efficient removal of nitrate and phosphate from wastewater. The iron nanoparticles synthesized by *E. crassipes*, *L. camara* and *M. pudica* were able to remove 74.52%, 71.12% and 65.23% of nitrate from the wastewater, respectively. However, the chemically synthesized iron nanoparticles removed 85.26% of nitrate. Although chemically synthesized iron nanoparticles remove a higher percentage of nitrate which is less stable as compared to the weed-based nanoparticles.

10.6 Conclusion

Recently, the biological methods can offer a competitive advantage over traditional chemical methods for synthesis of nanoparticles as they satisfy the principles of green chemistry. The biological methods open up new way for cost-effective, eco-friendly synthesis of nanoparticles by reducing use of chemicals and generating less waste. The weeds are responsible for various agricultural, environmental and economic losses. Hence, weed management and control remain a great challenge for the weed scientists. Several weed plants have been employed for the synthesis of metal nanoparticles that are suitable in several applications. Therefore, weed-based biosynthesis of nanoparticles is considered as an alternative strategy not only for the utilization of the invasive weed species but also for weed control through its harvesting and usage.

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