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Prospects of Fresh Market Wastes Management in Developing Countries

Springer

Water Science and Technology Library

Volume 92

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ISSN 0921-092X ISSN 1872-4663 (electronic)
Water Science and Technology Library
ISBN 978-3-030-42640-8 ISBN 978-3-030-42641-5 (eBook)
https://doi.org/10.1007/978-3-030-42641-5

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

The over increasing fresh market wastewater has associated with the growing concern over their fate and effects on the environment after being discharged or reused for irrigation. The disposal of fresh market wastewater into the environment and natural water system represents a serious worldwide problem due to the increased environmental awareness and stringent environmental standards governing which was set by different environmental protection agencies. The potential risks derived from the presence of pathogenic bacteria and organic compounds must be taken into consideration. The developments in the treatment technologies to produce high quality of fresh market wastewater is being devoted to safely reused.

The current book is an effort for researchers in the field of fresh market who believed that this is the right time to write a reference book to discuss on the current practice of fresh market wastewater management. The main target of this book is the academicians and researchers who would find more meaningful information on the management and recent treatment technologies of fresh market wastewater in the developed and developing countries.

This book aimed to discuss the proper management of fresh market wastewater. It provides a comprehensive review and referenced information on the fresh market wastewater in order to highlight its risk alongside the benefits. The book consists of 10 chapters. Chapter 1 describes the current management practices of fresh market wastes and thier impacts on environmental health. Chapter 2 addresses the applicability of natural coagulates for wastewater treatment. Chapter 3 reviews the application of nanotechnology in the inactivation of pathogenic bacteria in the wastewater. Chapter 4 focuses on the current situation for the fresh market wastewater treatment. Chapter 5 highlighted the production of microalgae in fresh market wastewater and its utilization as a protein substitute in formulated fish feed. Chapter 6 reviews microbial fuel cells as a green and alternative source for bioenergy production. Chapter 7 views the advantages for swim-bed technology in reducing seafood processing wastewater. Chapter 8 discusses biofilter aquaponic system for nutrients removal from fresh market wastewater. Chapter 9 highlights the struvite crystallization as an effective technology for nitrogen recovery in landfill leachate. Chapter 10 describes the qualitative characterization of healthcare wastes, this chapter included here gives more highlight for the wastewater with blood such as that generated from slaughterhouse wastewater.

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Chapter 1
Management Practices of Fresh Market Wastes and Impacts on Environmental Health

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Abstract The management practice of the fresh market wastes (wastewater and solid waste) plays an important role in the presence or absence of the adverse effect on the environment which received the disposed of wastes. The management process included the collection, segregation, transportation, treatment, and final disposal of the wastes into the environment. These processes aim to minimize the health risk associated with the fresh market wastes in terms of infectious agents. The health risk of the fresh market wastes is coming with the presence of pathogenic bacteria transferred from the fruits, vegetables as well as meats and fishes. The present chapter aimed to highlight the management role for reducing the infectious agents in fresh market wastes before the final disposal. It appeared that most of the infectious agents in fresh market wastes are pathogenic for plants. However, many of the infectious agents coming from meat and fishes might also cause some diseases for human. The main concern lies in the potential of fresh market wastewater to induce the eutrophication in terms of harmful algae blooms (HABs). However, the technologies used for the inactivation of HABs in the freshwater have been reviewed in this chapter.

Keywords Infectious agents · Health risk · Best practice · Disposal · Treatment · Harmful algae bloom

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A. A. S. Al-Gheethi et al. (eds.), *Prospects of Fresh Market Wastes Management in Developing Countries*, Water Science and Technology Library 92,
https://doi.org/10.1007/978-3-030-42641-5_1

1.1 Introduction

Fresh market wastes include the liquid and solid wastes generated from the fresh market centers. These wastes have several infectious agents generated from the vegetables, fruits, and meats. In terms of pathogenicity, the infectious agents from fresh market wastes have less pathogenicity for human except for those coming from meats. The low pathogenicity of the infectious agents from fresh market wastes is explained due to their optimal growth temperature where most of these organisms grow well at ambient temperature, while the optimal growth temperature for human is 37 °C. Nonetheless, some of the pathogenic bacteria such as *Salmonella* spp have high ability to infect a wide range of organisms from insects to human (Al-Gheethi et al. 2014, 2018).

On the other hand, the ability of the pathogenic organisms from fresh market wastes to cause an adverse effect on the environment as well as to reach the human via food chain depends on the persistence under hard environment conditions and survival for long time with active virulence factors. The Gram-positive bacteria have the survival factors which lie in their ability to form internal spores that increase their survival period under very hard environmental conditions. In contrast, the Gram-negative bacterial cells have a different mechanism which is used to prolong the survivability where many of Gram-negative bacteria can be converted from live or active to inactive state which is called viable but nonculturable (VBNC), this strategy takes place by minimize the anabolic and metabolic activity in the bacterial cells and thus reduces the requirements for the growth (Al-Gheethi et al. 2015, 2018).

The ability of the bacterial cells in the fresh market wastes to produce internal spores or to change from active state into the VBNC relies on the presence of inducers in the wastes such as the presence of toxic substances like chemical disinfectants or harmful irradiations which are used for the treatment for the fresh market wastewater. This is because these treatment processes have not led to the direct damage of the bacterial cells, rather than they act by inactivating one or more enzymes in the metabolic pathways in the bacterial cells. Therefore, the bacteria cells act by the alternative enzymes to change into VBNC or to produce the internal spores. Hence, the treatment of fresh market wastes represents the critical stage in the management process.

The current chapter aimed to discuss the management practice of the fresh market waste and their role in the reduction and minimization of the health risk associated with these wastes. Moreover, the recent technologies in activating harmful algae blooms (HABs) are reviewed.

1.2 Management Practice of Fresh Market Wastes

Five processes representes the management practice for the fresh market wastes; these processes included segregation (liquid and solid wastes), storage, transportation, treatment, and disposal.

1.2.1 Segregation Process

One of the best practices which contributes effectively in reducing the heal risk of the disposal fresh market wastes is the segregation process for the wastes before the storage and treatment process. The segregation here means to separate the solid wastes from the liquid wastes; the purpose of the segregation is to minimize the contact between microorganism and the solid wastes (Efaq and Al-Gheethi 2015). Indeed, the presence of solid materials in the wastewater might enhance the survivability of infectious agents, where the suspended solids in the wastewater play an important role in the colonization of the pathogen organism. Moreover, the presence of the suspended solids in the wastewater might also protect the pathogenic cells for the action of chemical and physical disinfection process (Efaq et al. 2015).

1.2.2 Storage Process

Huge quantities of the solid wastes are generated from the fresh market wastewater. These wastes are collected and then stored at a refuse room. In many of the developing countries, the wastes are piled on the platform outside the market leading to attract flies (Yhdego 1992). The storage process is a temporary stage in which the wastes are stored in closed room and then transferred into the treatment unit. During the storage period, series of the physical and chemical as well as biological reactions are taking place (Efaq et al. 2017). The main concern in the storage of the solid wastes of fresh market is the overgrowth of the fungi and bacteria due to the environmental conditions which support their growth. Therefore, the storage period of the fresh market solid wastes should not exceed 24 h (Al-Gheethi et al. 2013; Efaq et al. 2015).

1.2.3 Treatment of Fresh Market Solid Wastes

The fresh market solid wastes are rich in carbohydrates and proteins which are considered as a rich energy source for microorganism. Therefore, one of the best treatment options for these wastes is to produce organic fertilizers through the fermentation and composting process. The fermentation process can take place with presence or absence of free water (Bhargav et al. 2008). The process is used to convert the wastes with high contents of carbohydrates and proteins into useful organic fertilizers. In the fermentation, the carbohydrates are converted anaerobically into acids, alcohol, or gases via the hydrolysis enzymes produced by fungi, bacteria, and yeast (Bhargav et al. 2008). The fermentation process is leading to produce lactic acid, acetic acid, and butyric acid (Merfield 2012). Moreover, several factors should be considered for getting high fermentation efficiency. Among these factors, the temperature should be between 25 and 37 °C and pH between 4 and to 5.5 (Zulkeple et al. 2011). The temperature and pH factors affecting the fermentation process are based on their effect on the microorganism growth. The fungi have a wide range in their growth between 20 and 55 °C, while bacteria might grow at temperature between 10 and 40 °C. The extreme temperature and pH might affect negatively the microorganism metabolic process which in role effect the fermentation process. In the composition process, the organic materials are converted into carbon dioxide and water as a result of oxidation process via bacteria and fungi (Merfield 2012). The composition process is a natural process and takes place as a function of microorganism and thus the efficiency of this process relies on the environmental factors such as temperature and which induce or inhibit the microbial activity. The fermentation and composting of food waste is common practice in order to reduce the quantities of the food wastes. The process is not used only for the production of fertilizes but also aims to minimize the health risk associated with the presence of infectious agents as well as the odor.

1.2.3.1 Treatment Using Effective Microorganisms

Several techniques have been suggested for the treatment of food waste. However, EM technology which is used for treatment of different types of waste to convert them into useful by-product have been high interesting in the recent years (Kale and Anthappan 2012; Al-Gheethi 2015). EM is a consortium microorganism which includes lactic acid and phototropic bacteria as well as yeast (Higa 2001). The consortium is used to ensure an effective composting process within a short period (Higa 2001). The treatment of food waste leads to reduce the load of solid and liquid wastes. The composting period ranged from 2 to 20 weeks, however, with adding EM this period might be shorted to be within 1–2 days (Jamaludin et al. 2016). The composting process takes place aerobically and anaerobically. Therefore, the consortium microorganisms are used to have the ability to work under different conditions (Misra et al. 2003).

The composting method consisted of traditional and rapid composting methods (Misra et al. 2003). In the anaerobic decomposition, the materials are allowed to remain in the pit without turning and watering for three months while in aerobic decomposition. The composting process took place by itself without any additional element. In the large-scale passive aeration compost method windrow is used to mix the composting materials, to enhance passive aeration and to provide conditions congenial for aerobic decomposition. Composting operations may take up to eight weeks while active composting could range from 10 to 12 weeks. In the composting process, the EM solution is inoculated together into the food wastes and then incubated under the anaerobic process to allow fermentation to take place. The efficiency of this process relies on the presence of nitrogen and nutrients which induce the microbial growth, while the products are used as fertilizer.

1.2.4 Disposal Process

The disposal process represents the last stage of the management system of the fresh market wastewater. Since the food waste represents the highest percentage of the fresh market wastes and cause one of the large environmental problems. In many countries such as UK, the solid wastes such as food wastes are disposed of in landfill, via composting, or anaerobic digestion (AD). However, the new direction is to reuse these wastes as animal feeds, but this practice is currently illegal, due to the disease control concerns (Salemdeeb et al. 2017). Nonetheless, the applicability of the food wastes to be used as animal or aquaculture foods depends on the preparation, compositing, and whether to meet the standards or not. The main concern for the disposal of the food wastes into the landfill is the role of the landfill in producing large quantities of greenhouse gases (GHG) especially when the waste is subjected to the incineration (EC 2014). Food waste that is not handled properly can cause contamination of groundwater, emission of toxic gas, emanation of odor, and attraction of vermin.

1.3 Health Risk Associated with the Utilization of Food Wastes as Fertilizers

The application of fertilizers generated from the composting process of the food wastes is becoming more common due to these waste provided an alternative for the chemical fertilizers which have several of the disadvantages. However, the impact of the utilization of food fertilizers on the environment has not investigated in-depth yet. There are no reports on the infection agents or their disease have been transferred into the plants and animals. However, the potential of infectious agent transmission from food wastes to plants and animals is still possible. In a view for the nature of microbial load in the fresh market wastes, there are two types of infectious agents which include those coming from the vegetables and fruits and that coming from poultry and meat. The infectious agents from vegetables and fruits are growing at ambient temperature and might have an effect on the plants during the utilization of fertilizers generated from the compositing of fresh market wastes. However, here it should mention for the composting process which might lead to inactivating these infectious agents. The second type of infectious agents are those coming from animal and poultry which have the ability to grow at a temperature near to human temperature and thus represents a health risk for the animal and human. Besides, these organisms might survive during the composting process. Salemdeeb et al. (2017) compared four food waste disposal technologies in terms of 14 different environmental and health impacts. The study revealed that converting municipal food wastes into pig feed would have a lower environmental and health impacts than processing waste by composting or anaerobic digestion.

1.4 Harmful Algae Blooms (HABs) in Freshwater

A harmful algae bloom (HABs) is one of the natural phenomena which take place as a response for the contamination of the freshwater system with the nutrients (Davidson et al. 2016). HABs have been reported in several historical books and documents. However, in the recent years, HABs phenomenon has seen a significant spread due to the increase of human activities as the agriculture process, industrial systems, and the sewage disposal system into the natural water systems which led to the accumulation of nutrients in terms of nitrogen (N) and phosphorus (P) and contribute effectively in the HABs growth (Bae and Seo 2018). Moreover, the continuous changing of climate occupies a critical position in term of harmful algae proliferation where there are several methods to manage human activities, but climate changes could not be controlled which made the prediction of prevalence harmful algae the best option for the world (Wells et al. 2015; Chapra et al. 2017).

Three major strategies have been reported in the literature to treat or minimize HABs, include prevention (Kudela et al. 2015; Schindler et al. 2016; McPartlin et al. 2017; Jung et al. 2017; Lee and Lee 2018) mitigation (Paerl et al. 2016, 2018) and control (Hamilton et al. 2016; Noyma et al. 2016). The prevention strategy is used to manage and maintain the natural sources of water before the appearance and spread of HABs. The mitigation strategy is used, if the presence of the prevention and control strategies does not exist. While the control strategy is used in terms of chemical and biological as well as the flocculants which represent the most common and useful strategy around the world for reducing HABs (Fig. 1.1).

HABs represent dangerous phenomenon impacts on the environment by increasing the pollution level, change the quality of natural water sources, and establish a suitable environment which stimulates pathogenic bacteria (Wolf and Klaiber 2017). There are several syndromes poisoning caused by HABs, each of these syndromes has its own characteristics and impacts against aquatic organisms and humans (Grattan et al. 2016). HABs are algae organisms which have high potential to produce several toxins such as *hepatoxins*, and *neurotoxins*. The released toxins cause, diarrhea, vomiting, eye irritation, skin rashes, and respiratory symptoms (Villacorte et al. 2015). These symptoms are used as an indication for the infections since there are no specific diagnostic procedures for these toxins in human blood (Villacorte et al. 2015). HABs phenomenon in the water system in Malaysia has not investigated extensively. However, the algae growth in the natural water systems can be

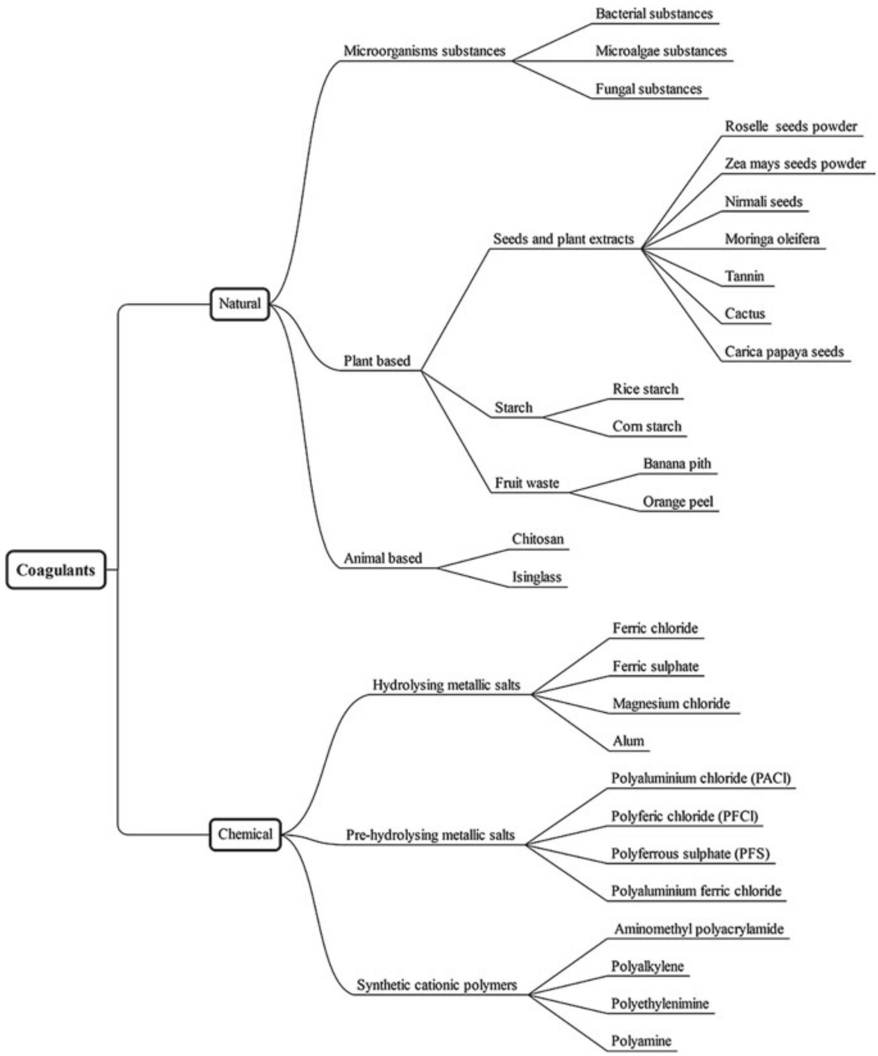

Fig. 1.1 Harmful algae blooms inactivation strategies

seen by the eyes. The utilization of these waters as drinking water resources represents real hazards for the human due to the presence of toxins. Darwish et al. (2016) revealed that the reverse osmosis (RO) technology which is an advanced treatment process for drinking water are not efficient for the removal of HABs toxins due to the high solubility and zwitterion properties of these substances. These properties allow the toxins to diffuse through the reverse osmosis membrane. The chemical and physical treatment methods of HABs have showed a high efficiency level of HABs reduction. However, they considered as contaminated sources and nontarget toxicity

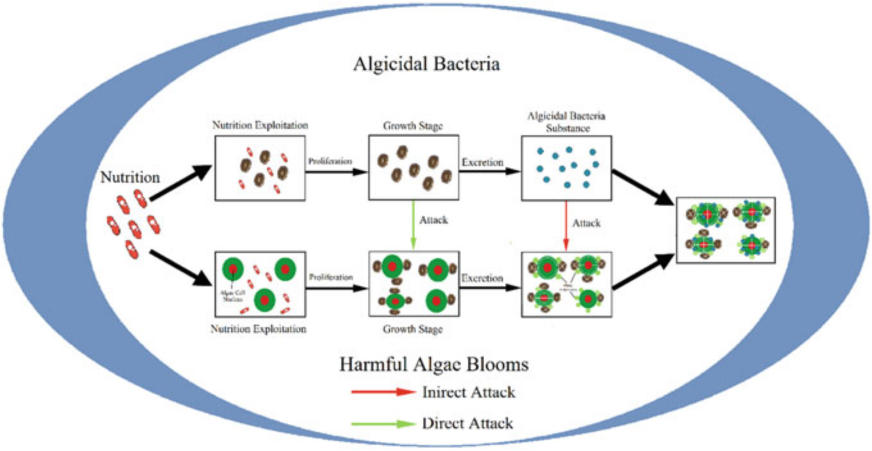

Fig. 1.2 The inactivation of harmful algae blooms by using algicidal bacteria

of water resources due to the chemical agents which caused a serious secondary contamination which leads to kill several types of aquatic organisms (Li et al. 2015a, b). In addition to the obvious disadvantages of the high cost of chemical and physical processes is the thing that made the world to resort to biological methods.

Several techniques are used to inactivate HABs, while the revolution of algicidal bacterium technique was widely used recently to overcome the harmful spread of algae blooms (Wang et al. 2016). The algicidal technique includes the microorganism algicidal strains mainly such as bacteria, fungi, and actinomycetes (Shu et al. 2017). The mechanisms of algicidal technique could be summarized in two different methods which are the direct close contact between the microorganisms and HABs cells (Sun et al. 2018) or the indirect contact which usually occurs due to the substances produced by the algicidal microorganisms (Hussain 2015) Fig. 1.2.

Myxobacteria were the first reported algicidal bacterium, which could kill unicellular and filamentous *Cladophora* on the premise of close contact between the *Myxobacteria* and the *Cladophora* (Mohr 2018). The bacteria are attached to the algal cells using their flagella based on the chemotaxis principle and then produced chitinase to degrade the algal cell walls, eventually leading to lyse and death of algae (Li et al. 2016). Most of algicidal bacteria cleave the algae cells by secreting algicides substances. However, the number of the identified algicides is much lower than the reported number of algicidal bacteria, which may be due to limitations in the identification techniques of algicidal compounds.

1.4.1 Control Methods of Harmful Algae Blooms

In the recent years, the wide proliferation of HABs has been observed in freshwater resources which posed global concerns. The outbreaks of HABs (*Cyanobacteria* blooms) in the lakes and rivers have increased the threats to human health and freshwater resources as well as the great economic damage due to the recreational water and aquaculture pollution (Smith and Daniels 2018). Several techniques and methods have been implemented to inactivate HABs cells (Table 1.1).

Table 1.1 indicates the chemical methods that included ozone, chlorine, chlorine dioxide, permanganate, or ferrate which have a high inactivation efficiency of HABs and it is estimated between 85.55 and 99.9999% (Naceradska et al. 2017; Visser et al. 2016), while physical methods included hypolimnetic aeration and oxygenation, hypolimnetic withdrawal and the flocculation of chitosan modified soil has been showed a lower efficiency compared to chemical methods estimated from 71.25 to 95.50% (Li et al. 2015a, b; Noyma et al. 2016). Both methods were very effective in terms of HABs treatment. However, they considered as contaminated sources and nontarget toxicity of water resources due to the chemical and physical agents which caused a serious secondary contamination which leads to killing several types of aquatic organisms (Li et al. 2015a, b).

In the recent years, science has seen a remarkable evolution in the biological treatment field, which is considered as environmental and economical alternative method to solve the water contamination issues around the world (Jin et al. 2017). Therefore, several researches were performed to investigate the efficiency of the biological method on HABs inactivation. Various biological strategies have been used to inactivate HABs including aquatic plants, aquatic animals, and algicidal microorganisms which recorded a high efficiency of HABs inactivation estimated between 88.60 and 99.9999% (Li et al. 2015a, b; Thees et al. 2019). While among these biological strategies, algicidal bacterium strategy became the most important strategy and researches focus. With a comparison to the physical and chemical strategies, algicidal bacterium-based strategy including algicidal bacteria, fungi, viruses, and protozoa are considered to be promising environmental methods due to the low contamination and hazardous effects on water sources, aquatic organisms and ecosystem (Backer et al. 2015; Harke et al. 2016).

1.4.2 Algicidal Activity of Bacteria on Harmful Algae Blooms

Several isolated bacteria species have the algicidal activity to inactivate the harmful fresh and marine water algae blooms. Table 1.2 displays the inactivation efficiency of algicidal bacteria species against various species of HABs under affected factors of inactivation process.

Table 1.1 Inactivation methods of HABs

Treatment method	Treatment type	Required material/Process of treatment	Removal rate (%)	Ref
Chemical methods	Preoxidants	Ozone, chlorine, chlorine dioxide, permanganate, or ferrate	85.55–99.9999	Naceradska et al. (2017)
	Algaecide	copper sulfate addition ($CuSO_4 \cdot 5H_2O$)		Visser et al. (2016)
	Inorganic coagulants	Clay coagulants		Huh and Ahn (2017)
Physical methods	Hypolimnetic aeration and oxygenation	Inject or increase the dissolved oxygen of water to reduce phosphate (P)	71.25–95.50	Visser et al. (2016)
	Hypolimnetic withdrawal	Reduced the bottom water residence time, provide nutrients to the epilimnion by limited vertical mixing		Visser et al. (2016)
	Flocculation	Ex, flocculants and phosphorus adsorbing natural soil and modified clay, chitosan modified soil		Li et al. (2015a, b), Noyma et al. (2016)
Biological methods	Algicidal bacteria	Using bacterial species	88.60–99.9999	Shu et al. (2017)
	Biodegradation of organisms	To biodegrade the metabolites of *Cyanobacteria* by using organisms Ex. *Rhodococcus wratislaviensis* DLC-cam to biodegrades MIB metabolite and *Stenotrophomonas* sp, to biodegrades *geosmin metabolite*		Li et al. (2015a, b), Thees et al. (2019)

Table 1.2 Effect of algicidal bacteria species against various species of HABs, (A comparison between the previous studies)

Algicidal bacteria	Bacterial density (Cell mL^{-1} or cfu mL^{-1}); Liquid dosage, (mL)	HABs	Algae density (Cell mL^{-1} or cfu mL^{-1}); Chlorophyll-a, (µg mL^{-1}); Liquid dosage, (mL)	Algae source	PH value	Shaking speed (rpm)	Inactivation Time (h)	Temp (°C)	Removal ratio, %	Ref
Bowmanella denitrificans	1×10^7 cells mL^{-1}	Chlorella vulgaris	1.5×10^4 cells ml^{-1} 7.5×10^4 cells ml^{-1}	Seawater	7.2	180	24	30	99.00	Jiang et al. (2014)
Bacillus sp.	1.9×10^7 cfu mL^{-1}	Microcystis aeruginosa	0.56 µg mL^{-1}	Freshwater	8.2	80	120	28 ± 1	99.9999	Shao et al. (2014)
Aeromonas sp.	2.1×10^9 cfu mL^{-1}	Microcystis aeruginosa	4.6×10^6 cells ml^{-1}	Freshwater	7.2	150	96	30	99.9999	Nishu et al. (2019)
Enterobacter sp.	1/100 mL of Algae cultures ($D = 1 \times 10^7$ cfu mL^{-1})	Scenedesmus	1×10^6 cfu mL^{-1}	Freshwater	7.5	NR	480	25	75.00	Liao and Liu (2014)
Streptomyces sp.	3.5 mL	Phaeocystis globosa	100 mL	Seawater	5, 9	150	48	28	97.70	Zhang et al. (2015)
Pseudomonas fluorescens	5×10^6 cells mL^{-1}	Stephanodiscus hantzschii	9×10^5 cells mL^{-1}	Freshwater	7	NR	120	15	90	Jung et al. (2008)

According to Table 1.2, there was a confirmed high efficiency of algicidal bacteria to inactivate HABs, where the previous studies showed a removal ratio of HABs estimated between 75 and 99.9999%. Furthermore, the bio-algicidal treatment method was employed in both marine and freshwater to investigate the algicidal activities of different isolated bacteria and algal blooms species. Algicidal bacterium is one of the most common biodegradation methods of HABs at the laboratory scales. However, there are urgent needs to investigate and assess the algicidal activities and efficiency in the large scale as well as the toxicological and health effect studies are compulsory before large-scale implementation (Ge et al. 2014). The investigation of algicidal activities of bacteria will lead to identify a clear concept of the algicidal bacteria mechanisms and specify the factors affecting the inactivation process.

1.4.3 Inactivation Mechanisms

Surviving competition may be the main mechanism for the bio-control of HABs. To survive, various allelochemicals are secreted by competing microorganisms (algicidal bacteria/fungi, plankton, aquatic plants, etc.) against algae to fight for light, nutrient substances, living spaces (Uronen et al. 2007). Some of bacteria strains inhibit the bloom-forming growth of Cyanobacteria in highly eutrophic lakes, through the production of *allelochemicals* (Li et al. 2015a, b). According to Eckersley and Berger (2018), the inactivation of HABs by algicidal bacteria was investigated by using Scanning Electron Microscopy (SEM), where the study has showed the difference of *M. aeruginosa* cells under the SEM before and after the inactivation process which occurred due to the algicidal activities of bacteria. HABs cells mostly have a spherical shape which contain the cell wall and nucleus. The study considered that any changes on the spherical shape of HABs cells indicate inactivation or deformation of HABs cells Eckersley and Berger (2018).

1.5 Conclusion

Fresh market wastewater needs an advanced environmental management system to minimize the health risk associated with the infectious agents. Storage period represents the critical point in the management system, among factors which contribute to the increase of infection agents risk is the temperature between 25 and 37 °C and pH between 4 and 5.5. Therefore, the storage of fresh market wastes should not be more than 24 h. Moreover, the treatment technologies should also be focused on removing the nutrient to prevent the occurrence of eutrophication and harmful algae blooms.

Acknowledgements The authors would like to thank the Ministry of Education Malaysia for providing FRGS vot K090 (An Insight Of Xenobiotic Organic Compounds) as a financial support for this research project.

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Chapter 2
Natural Coagulates for Wastewater Treatment; A Review for Application and Mechanism

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Abstract The increase of water demand and wastewater generation is among the global concerns in the world. The less effective management of water sources leads to serious consequences, the direct disposal of untreated wastewater is associated with the environmental pollution, elimination of aquatic life and the spread of deadly epidemics. The flocculation process is one of the most important stages in water and wastewater treatment plants, wherein this phase the plankton, colloidal particles, and pollutants are precipitated and removed. Two major types of coagulants are used in the flocculation process included the chemical and natural coagulants. Many studies have been performed to optimize the flocculation process while most of these studies have confirmed the hazardous effects of chemical coagulants utilization on the ecosystem. This chapter reviews a summary of the coagulation/flocculation processes using natural coagulants as well as reviews one of the most effective natural methods of water and wastewater treatment.

2.1 Introduction

Human activities and the over population are among the major reason for environmental pollution, where an enormous amount of natural water is employed to serve the agricultural, industrial and other activities, which in turn produce tons of contaminated wastewater. Huge quantities of untreated wastewater are disposed of directly to natural water resources in many low and middle-income countries, which cause environmental and health issues such as the prevalence of the harmful algal blooms,

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A. A. S. Al-Gheethi et al. (eds.), *Prospects of Fresh Market Wastes Management in Developing Countries*, Water Science and Technology Library 92,
https://doi.org/10.1007/978-3-030-42641-5_2

which can disrupt treatment and water refining systems and outbreaks of deadly epidemics such as Cholera epidemic (Al-Gheethi et al. 2018; Wurtsbaugh et al. 2019; Williams et al. 2019). Wastewater of textile, slaughterhouses, and chemical industries that receive high attention from researchers are the most common sources of natural water contamination due to its high content of organic and inorganic matters and low degradability under the natural conditions in addition to its unique characteristics in developing the bacterial strains and viruses (Deghles and Kurt 2016; Pahazri et al. 2016). Over the past years, many techniques have been used to treat various types of wastewater, including physical and chemical biological techniques, which still have a high performance on wastewater treatment. However, there have been many issues such as the high cost of chemical processes, the secondary pollution resulted from the excessive utilization of chemicals and the high production of industrial and domestic wastewater that have forced researchers to find alternative treatment methods for these techniques to achieve the optimal efficiency of environmental treatment and control the massive quantities of generated wastewater. Global emphasis on environmental conservation and the strict regulations have forced researchers and many countries to develop the wastewater treatment systems to comply with the global standard where many studies and applications have been performed on the alternative utilization of the natural coagulants for the flocculation process of wastewater which have shown high efficiency in terms of processing cost and treatment performance comparing to the chemical coagulants. The chapter reviews a summary of the coagulation/flocculation processes using natural coagulants as well as reviews one of the most effective natural methods of water and wastewater treatment.

2.2 History of Coagulants Utilization

The utilization of coagulants in wastewater treatment is an ancient process goes back thousands of years, and it has developed throughout the ages until sophisticated utilization of the coagulation processes were achieved during the last century when ferric chloride and aluminium sulphate were used as coagulants widely in wastewater treatment plants. The theory of particle collision function was developed in 1917, which was considered as a reference to explain the concept of the changes in the number of particles in the flocculation process, this initiative came after Schultz–Hardy suggested to clarify the mechanism of coagulation. In 1928, Mattson (2002) discovered that the aluminium and iron hydrolysis products were more important than the trivalent ions themselves. Several studies have been conducted in 1934 to investigate the effects of pH and the diversity of anions on the floc formation time. The following studies focused on finding better coagulants and on the mechanical methods of producing better flocs. Langelier and Ludwig (1949) have created a new coagulation theory relied on two distinct mechanisms to remove the colloidal impurities which are the double-layer compression and precipitate enmeshment. Double-layer compression mechanism known as the process of agglomerate and precipitate resulting from the domination of particles over the repulsive forces, while the precipitate enmeshment is the mechanism where the colloidal particles are physically enmeshed (entrapped) by metal precipitates when they are forming and settling. These mechanisms have been elaborated by scientists in 1963 who proposed to name the mechanism of double-layer compression by "coagulation" and the mechanism of precipitate enmeshment by "flocculation". In 1980 systematic studies have been conducted to improve the coagulation performance of natural organic materials (AWWA Research Committee Report 1989), various surface waters have been used during these studies and several variables were investigated, such as mechanical conditions of coagulation, pH, temperature and other factors. While the coagulation has continued to develop using various methods to this day.

2.3 Natural and Chemical Coagulants

The coagulants are divided into chemical and natural coagulants as shown in Fig. 2.1. The coagulation process is one of the most effective treatment processes for removing total suspended solids (TSS), chemical oxygen demand (COD), organic materials, and colours. However, the performance of the removal process depends on the type of the coagulant used. The general concept of the coagulation process can be defined as the neutralization of the suspended matters with the negative charge and agglomerate the destabilized particles to form a heavy clumped mass of small particles that allow them to precipitate (Yusoff et al. 2018).

Aluminum salts and poly-aluminium chloride (PAC) are the most used coagulants in the treatment of water and wastewater all around the world, as they are considered widely available chemical coagulants which have high efficiency of treatment. The reason that led to involve it in most of the wastewater treatment processes recently and dispensed with traditional treatment methods of the natural coagulants (Zhao et al. 2015). However, recent studies performed by researchers have raised doubts about the danger of introducing these substances into the environment, where alum-based coagulants leave a high level of aluminium residuals in the treated water under the conditions of cold temperature or low pH levels which can damage the ecosystem and cause health issues (Matilainen et al. 2010).

In addition to that, the long-term exposure to aluminium residuals may cause bioaccumulation in the human brain, bones, and liver (Walton 2012). Furthermore, low molecular weight Al species can pose a deadly threat to the organisms due to the high solubility and zwitterion properties of these substances which enable them to penetrate biological membranes (Trenfield et al. 2017; Yusoff et al. 2018).

On the other side, ferric salts and synthetic polymers coagulates have been employed to replace the alum-based coagulates but the limitation of the availability and the lack of environmental impacts studies about these coagulates in addition to the high cost of them made their success limited (Ndabigengesere et al. 1995). In view of the hazardous caused by the utilization of chemical coagulants, the return to the natural coagulants application has become a desirable option (Verma et al. 2012). The applications of natural coagulants can be a suitable option for water

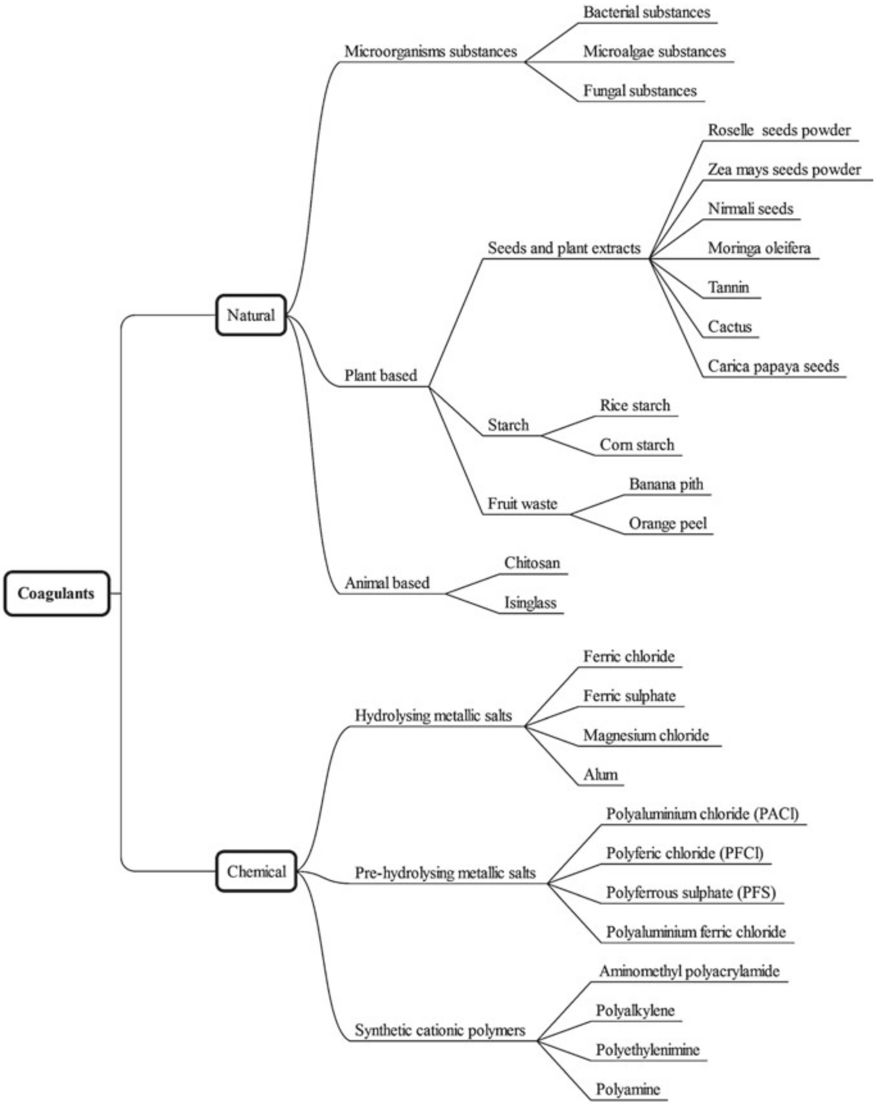

Fig. 2.1 Coagulants categories

and wastewater treatment in the future where many studies have been conducted to develop these coagulants to provide a high performance of water and wastewater treatment where. Natural coagulants include extracts of microorganisms, animal or plant, seed extracts, and other sources (Fig. 2.1), where the chitosan coagulant consider one of the most natural promising materials in the coagulation process (Renault et al. 2009). Natural coagulants distinguish as safe for wide utilization, environmentally friendly and inexpensive, in addition to their high efficiency in the removal of organic matters, dyes, and other wastes in water and wastewater. Many researches have been conducted to assess the performance of natural coagulants comparing to the chemical coagulants. Previous literature has indicated that the efficacy of natural coagulants is approximately equal to that of chemical coagulants, where according to the comparison between the performance of the natural and chemical coagulants in Table 2.1.

The natural coagulation of chitosan has recorded a higher removal ratio of COD comparing with the alum in the textile wastewater which estimated by 70.5 versus 36%, and with the ferric chloride by 70.5 versus 68.75% (Nechita 2017; Naghan et al. 2015). Moreover, natural coagulants have overcome chemical coagulants in the removal efficiency of total suspended solids (TSS), while previous studies showed that there is a convergence between natural and chemical coagulants in the high-performance removal of oils and greases from wastewater (Hosny et al. 2016; Sun et al. 2017). The increase in coagulants may lead to an increase in the efficacy of water and wastewater treatment, but chemical coagulants cannot be used excessively to avoid secondary pollution and the hazards that can be resulted from this excessive utilization, in contrast to using a high dose of natural coagulants which is safe and highly efficient. A previous study has been performed by Shan et al. (2017), showed that the high dosage of natural coagulants have the high potential to remove heavy metals from wastewater.

2.4 Coagulants/Flocculants Mechanism

The coagulation and flocculation mechanisms differ according to the coagulants type, as there are four types of coagulation/flocculation processes including charge neutralization, sweep coagulation, bridging and patch flocculation (Amran et al. 2018) (Fig. 2.2). The stabilized colloidal dispersion undergoes to high concentrations of simple salts in the coagulation process, the added counter-ions permeate to the diffuse double layer, and this process compresses the double layer and lead to the reduction of the repulsion among colloids which allow the aggregation by van der Waals forces (Liu et al. 2015). According to Schulze-Hardy rule, the effect of this phenomenon can be stronger by the increase of the counter ions charge where the previous literature showed the vary of the relative power of Al^{3+}, Mg^{2+}, and Na^{+} for the coagulation of negative colloids estimated in the ratio of 1000:30:1 (Ghernaout et al. 2011; Trefalt et al. 2017). Charge neutralization mechanisms usually employ the *ionisable polymer* (*polyelectrolytes*) coagulants in the stabilization process of colloidal particles. The colloidal particles are negatively charged particles due to the repulse process between them. The added coagulants with a high positive charge take advantage of the surfaces of negatively charged colloids to be stick on them (Surface absorption) in the coagulation/flocculation processes which leads to the coagulants penetration phase to the diffuse double layers surrounding by the particles rendering them heavier and smaller in volume due to the closer distance between the particles and end to the accumulation (Kristianto 2017). The coagulants with high charge

Table 2.1 Natural and chemical coagulations performance

Coagulant type	Wastewater type	Coagulant dosage	Treatment efficiency in terms of parameter removal (%)	Reference
Natural coagulants				
Moringa Oleifera Seed	Landfill leachate	30,000 mg/L	Fe (100%), Cu & Cd (98%), Pb (78.1%), respectively	Shan et al. (2017)
	Domestic wastewater	200 mg/L	Turbidity (90.43%), BOD_5 (55.77%), Phosphorus (88.84%), Nitrogen (54.54%), Calcium (62.38%), $CaCO_3$ (50.48%), Coliform (97.5%)	Ugwu et al. (2017)
Chitosan	Textile wastewaters	10000 mg/L	TSS (99.65%), COD (70.5%), Sulphides (25.53%), Greases and oil content (79.57%)	Nechita (2017)
	Oily produced water	Unit weight	Oil (96.35%)	Hosny et al. (2016)
Tamarind seed powder	Detergent wastewater	400 mg/L	COD (43.50%), Turbidity (97.78%)	Ronke et al. (2016)
Orange peel	Dairy wastewater	200 mg/L	Turbidity (97%)	Anju and Mophin (2016)
Rice starch	Microalgae sample	120 mg/L	Microalgae removal (80%)	Choy et al. (2016)
	Kaolin wastewater	120 mg/L	Kaolin removal (50%)	Chua et al. (2019)
Chemical coagulants				
Ferric chloride	Industrial wastewater	150 mg/L	COD (68.75%)	Naghan et al. (2015)
	Metalworking wastewater	50–1000 mg/L	COD (55–93%), TOC (41–77%)	Demirbas and Kobya (2017)
Alum	Textile wastewater	550 mg/L	Dye (75%), COD (36%)	Naghan et al. (2015)
	Metalworking wastewater	50–1000 mg/L	COD (65–97%), TOC (49–81%)	Demirbas and Kobya (2017)
Ferric sulphate	Metalworking wastewater	50–1000 mg/L	COD (43–92%), TOC (36–76%)	Demirbas and Kobya (2017)

(continued)

Table 2.1 (continued)

Coagulant type	Wastewater type	Coagulant dosage	Treatment efficiency in terms of parameter removal (%)	Reference
Aluminium chloride	Metalworking wastewater	50–1000 mg/L	COD (48–96%), TOC (38–80%)	Demirbas and Kobya (2017)
Polyaluminum chloride	textile wastewater	30 mg/L	COD (44.75%) BOD_5 (44.42%) TS (72.27%) TSS (68.53%) Dye (45.80%)	Bazrafshan et al. (2016)
Polymeric aluminum ferric silicate	High-oil-containing wastewater	60–120 mg/L	COD (98.2%), Oil (98.4%)	Sun et al. (2017)
Poly-aluminum chloride	Landfill leachate	400 mg/L	BOD5 (67.5%), COD (69.3%), Total Kjeldahl nitrogen (63.6%), Total phosphorus (73.1%), TSS (81.3%)	Balarak et al. (2018)

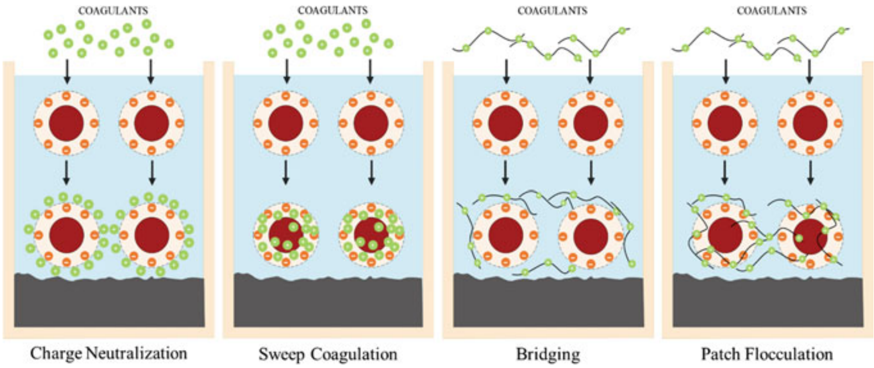

Fig. 2.2 Coagulants/flocculants mechanisms at treatment process

density and low molecular weight can enhance coagulation performance as at the optimal dosage of coagulant, there is a potential to get zero zeta (Bolto and Gregory 2007). In the sweep coagulation, a high concentration of metal salt coagulant is added to the water which causes the precipitation of amorphous hydroxide ($M(OH)_3$), where the precipitated ($M(OH)_3$) act as a mesh to precipitate the colloid particles (Nan et al. 2016). The mechanism of the bridging flocculation can be defined as the adsorbing of a segment of a polymer chain several particles lead to gather them together. In this coagulation type, the segments of polymer chains absorbed on other particles through the sufficient unoccupied surface of the particles (Bolto and Gregory 2007). While in the patch flocculation, the oppositely charged particle surface adsorb the coagulants to provide a non-uniform distribution of the surface charge (Suopajärvi 2015).

2.5 Natural Coagulants Plants-Based

Natural coagulants are usually divided into cationic, anionic or nonionic, and hence are also termed as polyelectrolytes (Saranya et al. 2014). The cationic polymers are many such as chitosan and cationic starches, as well as the anionic polymers such as sulphated polysaccharides and modified lignin sulfonates. The starch and cellulose derivatives are considered as non-ionic natural polymers (Freitas et al. 2018). The natural coagulants application are always associated with the mechanisms of charge neutralization and bridging flocculation where the long chain of their structures increase the number of empty adsorption sites (Wurochekke et al. 2016). These two mechanisms provide the main principles to the inner operations of the plant-based coagulants (Saleem and Bachmann 2019). Many natural coagulants/flocculants have used in the previous literature and applications, only four types are commonly known within the scientific community which is *nirmali* seeds (*Strychnos potatorum*), *Moringa oleifera, tannin* and *cactus* (Vijayaraghavan et al.

2011; Atiku et al. 2016; Rosmawanie et al. 2018). Three general phases are used to produce the natural coagulants (Plant-based) include the primary phase (pulverizing), secondary phase (extraction) and tertiary phase (purification) (Yin 2010). In the first phase, the unwanted and unused parts of the plants or fruits are removed while the targeted parts usually undergo a traditional drying method then pulverizing to be in powder form. The second and tertiary phases have been suggested to prepare the powder formed at the first phase (pulverizing), where these powder not only contains the coagulating active agents but also plant tissues and organic matters which may contaminate the water rather than treat it (Ghebremichael et al. 2005). In the second phase (extraction), the coagulating agents can be extracted from the pulverized powder using various solvents such as water, organic, and salt solution. The availability and low cost of water make it the best option of the extraction of coagulating agents, however, several studies have been reported that the salt solution (NaCl) is more affected than water in extraction performance, where the results have shown that the extracted coagulants by NaCl were more effective in coagulation activity with a dosage estimated by 7.4 times less than the extracted coagulants by distilled water to remove the kaolinite turbidity. Tertiary phase (purification), is usually a lab-scales and academic researches phase due to its high cost. The previous studies have been suggested several methods for the purification process such as lyophilization (Grossmann et al. 2018), ion-exchange (Megersa et al. 2019) and dialysis (Idris et al. 2016) which are considered as effective methods of purification of extracts (especially for *M. oleifera*) and they can be incorporated into a scaled-up set up for treatment of higher throughput of turbid water.

2.5.1 Nirmali Seeds

S. potatorum (*nirmali*) is a type of tree that grows in southern central parts of India, Sri Lanka and Burma with a moderated size. The Indian books of Sanskrit have reported the effective use of *nirmali* seed to treat the turbid surface water over 4000 years ago (Yin 2010), where it considered as the first plant-based coagulant for the water treatment. The extracts of *nirmali* seed are anionic polyelectrolytes, where these extracts work on the destabilization of particles in water by the means of interparticle bridging. The contents of the seed extracts include lipids, carbohydrates and alkaloids containing the −COOH and free −OH surface groups which enhance the performance of the extracts coagulation (Vijayaraghavan et al. 2011).

2.5.2 Moringa Oleifera Seed

M. oleifera (horseradish or drumstick tree) one of the tropical plants that grow in India, Asia, Sub-Saharan Africa and Latin America. *M. oleifera* seed is one of the most common natural coagulants in environmental science where it contains water-soluble substance and edible oil (James and Zikankuba 2017; Hauwa et al. 2018). *Moringa* parts have been used in many applications such as food and medicinal in the rural areas (Gopalakrishnan et al. 2016), but this point will discuss the usage of the *Moringa* seed extracts as coagulants. It has been reported that the *M. oleifera* seed extracts were used in some countries to treat the river water turbid (Hoa and Hue 2018). Furthermore, many recent and ancient studies have emphasized the natural coagulating characteristic of the *M. oleifera* seed extracts (Petersen et al. 2016; Sulaiman et al. 2017). *M. oleifera* coagulation depends on the mechanism of charge neutralization (Al-Gheethi et al. 2016). Various suggestions and research have been provided to specify the coagulating agent of *Moringa*, while most of these researches agreed that the active agent is cationic protein and it has been suggested that the cationic protein molecular mass of 12–14 kDa with an isoelectric point (pI) between 10 and 11 (Camacho et al. 2017). On the other hand, Okuda et al. (2001), reported that the active coagulating agent from the aqueous salt extraction is not a protein, polysaccharide or lipid but an organic polyelectrolyte with a molecular weight of approximately 3.0 kDa. As a concussion, the coagulation mechanism of the *M. oleifera* seed extracts can be defined as the interaction process between the negatively charged colloids particles at the contaminated water and the positively charged protein molecules in the active ingredients (Camacho et al. 2017).

2.6 Natural Coagulants Animal-Based

Animal-based coagulant/flocculants is one of the most environmentally benign and less expensive coagulants/flocculants, where it is an effective polysaccharide employed to remove the water contaminants such as the Chitosan.

2.6.1 Chitosan

Chitosan is one of the most widely used coagulants among the animal-based coagulants. Chitosan is an amine-based polysaccharide that is derived via deacetylation of chitin and considered as a biopolymer. The chitin is the second most abundant polysaccharide in nature after cellulose. Chitosan has been used as a single-component coagulant in different studies and applications, such as the settling of clay suspensions (Ferhat et al. 2016), water turbidity and color removal (Momeni et al. 2018), and the treatment of industrial wastewater (Negm et al. 2015). Many studies have been reported that chitosan coagulants depend on the charge neutralization mechanism while there are other studies reported that inter-particle bridging occurs in conjunction with charge neutralization (Fig. 2.3). However, Li et al. (2013) has been suggested to use the patch mechanism because of the high concentration of the positive charges on the chitosan compared to the negative charges in the water

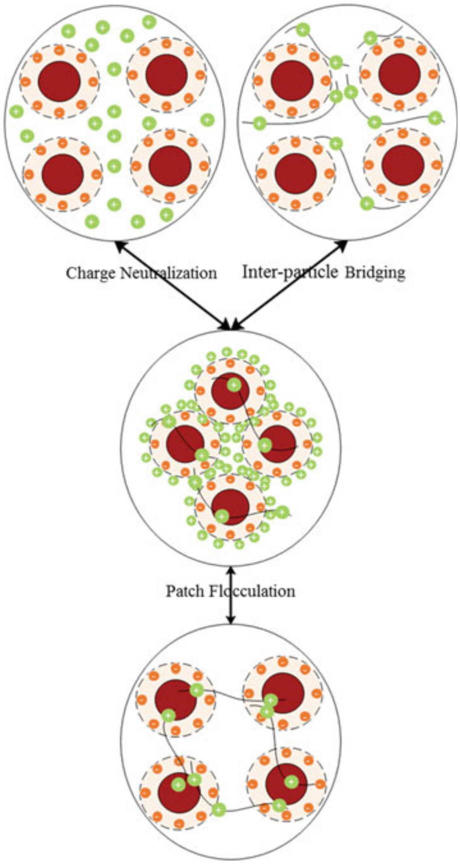

Fig. 2.3 Chitosan coagulation/flocculation mechanisms

where neutralization occurs in smaller regions in a step-wise fashion, thus leading to patches of charge neutralization (Fig. 2.3).

2.7 Natural Coagulants Microorganisms-Based

Microorganisms such as bacteria, fungi, microalgae have been widely used in wastewater treatment and organic matter degradation as well as to produce coagulants/flocculants with different structures and mechanisms. The produced substances from the microorganisms are considered environmental and green substances and have a high performance of coagulating/flocculating the colloids particles and other particles with an activity reach 90% depending on the microorganism strain and the operating conditions (Ben Rebah et al. 2018). This type of treatment is usually called bio flocculation, due to its dependence on the interparticle bridging mechanism (Shahadat et al. 2017). Microorganisms produce bioflocculants as metabolites of their cellular growth. The produced bioflocculants are extracellular polymers comprise complex multi-chains, heavy molecular polymers with repeating units of branched sugar derivatives, glycoprotein, and polyols which formed during the microorganism growth (Pathak et al. 2015). The extracellular polymeric substances (EPS) of microorganisms are produced generally intracellularly and exported outside the cell wall except in some microbial strains such as levans and dextran which synthesis and polymerize the EPS extracellularly. EPS have shown both the negative and positive effect on the wastewater and sludge where it depends on the net negative and positive ions (Sheng et al. 2010). EPS used the ion exchange mechanism in the flocculation process where a study has been performed in heavy metal adsorption using EPS found that Ca^{2+} and Mg^{2+} were released into the solution simultaneously (Yuncu et al. 2006). EPS are negative in charge which enables these compounds to absorb the positively charged particles at the water and wastewater through electrostatic interaction.

2.8 Conclusion

It can be concluded that the natural coagulants (Plant, animal or microorganism) have high applicability in the removing of wastewater pollutant more efficiently and safer than the chemical coagulation which has many of the disadvantages as such carcinogenic properties.

Acknowledgements The work was supported by GERAN INDUSTRI (M003) (Kajian Penilaian Alam Sekitar Untuk Parlimen Simpang Renggam).

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Chapter 3
Bio-nanotechnology Application in Wastewater Treatment

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Abstract The nanoparticles have received high interest in the field of medicine and water purification, however, the nanomaterials produced by chemical and physical methods are considered hazardous, expensive, and leave behind harmful substances to the environment. This chapter aimed to focus on green-synthesized nanoparticles and their medical applications. Moreover, the chapter highlighted the applicability of the metallic nanoparticles (MNPs) in the inactivation of microbial cells due to their high surface and small particle size. Modifying nanomaterials produced by green-methods is safe, inexpensive, and easy. Therefore, the control and modification of nanoparticles and their properties were also discussed.

Keywords Bio-nanoparticles · Plant · Application · Antimicrobial activity

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https://doi.org/10.1007/978-3-030-42641-5_3

3.1 Introduction

Recent advancement of nanotechnology aimed to use the material at the atomic and molecular levels to reach new nanometre-scale materials (Cheng et al. 2015). Many researchers focused on the major properties of nanomaterials such as surface morphologies, crystalline and molecular structure, porosity, electric and magnetic properties as well as solubility of the particles, all these form and assembly properties are due to their small shape and size less than 100 nm (Fan and Zhang 2016). Nanomaterials properties are sensitive toward sizes because the number of atoms on the surfaces is identified by the sizes (Yashni et al. 2019a). The changes in sizes change the number of atoms at the surfaces and thus changes the properties of nanomaterials drastically compared to their bulk properties (Boysen and Muir 2016). The properties of nanoparticles change due to changes in chemical composition and particle size (Suresh 2014). The product obtained from the synthesized nanoparticles is fascinating and observed different properties of the particles, including physical and chemical properties, because of the quantitative effect and harmony of energy levels, the proportion of atoms is higher on the surface of the nanomaterials due to increase in surface area versus the volume ratio of nanoparticles (Hulkoti and Taranath 2014). There are other noteworthy properties like crystalline nature for understanding elasticity and strength of materials, which basically correspond to different atoms' and molecules' structural arrangement, increased catalytic properties such as high surfaces for chemical reaction and high electrical conductivity in the nanomaterials (Kumar et al. 2012).

The unique properties, nanoparticles and nanomaterials offered have attracted the interest of many researchers, especially in the fields of biomedicine, food and feed, health care, drug delivery, environment, optics, light emulsions, health, cosmetics, aerospace, mechanical, chemical industries, electronics, and energy sciences (Korbekandi and Iravani 2012). The changes in the sizes of nanoparticles created new nanostructured materials that give unique properties within the nanometre area that depends on the atom numbers that could form nanoclusters, rods, rings, or dots (Ananya 2012). Many researchers continued to follow these trends of fabricating nanomaterials including rare materials for the past years (Sun et al. 2000), directing to development of nanotechnology field (Bréchignac et al. 2008).

Nanotechnology is mainly focused on the study of the formation, manufacture, modification, and application of Nanosize materials that have at least one external dimension within a range of size from 1 to 100 nm (Khan et al. 2017). In the past, for reducing the size of components electronic devices were needed. However, after the advent of the science of nanotechnology, many researchers apply and use this technology in various fields such as chemistry, physics, biology, and other natural sciences (Mobasser and Firoozi 2016). Currently, nanotechnology has also been applied in many aspects of life, such as clothing (Almeida and Ramos 2017); architecture (Bovi et al. 2017); and cosmetics (Raj et al. 2012). Nanotechnology has the ability to develop materials which can be applied in the manufacturing of medical instruments and water purification tools, and it has also enhanced the development of small-scale devices that are required in the area of medicine and vision service. Furthermore, nanotechnology applications have gained widespread acceptance in the fields of medicine, energy, biotechnology, agriculture, and environment (Saranyaadevi et al. 2014).

Development of nanomaterials researches focuses on the synthesis, modification, and characterization of nanomaterials (Wang 2000). Nanomaterials can be divided into two main types; the first type is all three dimensions within a nanometre range (1–100 nm) called nanoparticle, maybe polymer, metallic, or metal oxide (Rotello 2004; Gubin 2009). The second type has at least one dimension within the nanoscale range and is called nanostructure (Han et al. 2012). They can be one external dimension (1D) that is out of 100 nm range, such as nanorods and nanowires or two external dimensions (2D) like Nano sheets and Nano films (Cao 2004).

Synthesizing of nanomaterials is divided into two approaches, the "top-down" and the "bottom-up" approach. Top-down, where the size of the material is reduced to obtain smaller materials started from bulk materials and processed through physical and chemical methods. The synthesis from bottom-up is called self-assembly, where materials are formed by assembling atoms or by clustering molecules to obtain bigger nanomaterials. Nanoparticles have been prepared in the past years, using physical and chemical methods such as thermal decomposition, laser evaporation, chemical and physical vapour deposition (Iravani et al. 2014). The hazardous reagents, maximum requirements are often involved in chemical and physical methods. High-density lasers are used to evaporate the atoms and then cool them to form nanoparticles; high-temperature helium gas and radiofrequency plasma are also used to produce nanoparticles. The silver nanoparticles were prepared using the chemical method, where silver salt was used as precursor and sodium borohydride as a reducing agent, colloidal solution as a stabilizing agent (De Sio et al. 2015).

Recently, nanoparticles are produced by chemical and physical methods. These methods produce nanoparticles in large quantities in a short time, but these methods require high voltage, high temperatures, and use of non-degradable materials (Kruis et al. 2000). The above methods are costly, require high energy, and produce a toxic waste that can harm the environment in general and humans in particular. The use of these products may be limited to different applications (Mahdieh and Fattahi 2015). Today, there is a new approach that uses biological sources instead of chemical and physical methods in producing nanoparticles, where the production process is used from (bottom-up), and this is a new field that contributes to the development of nanoscience and is called bio-nanotechnology (Singh et al. 2016; Yashni et al. 2019b). Biotechnologies have become more attractive because they are environmentally friendly, do not consume too much energy, and have high efficiency in producing nanoparticles with unique properties (Kulkarni and Muddapur 2014). Also, biogenic sources of plants (Njagi et al. 2010) and microorganisms (Hulkoti and Taranath 2014) have shown their potential extensively in the green synthesis of nanomaterials and have many applications. Plants possess different leaves that are widespread on the ground. This includes large and small sizes and different colours, some are considered as food for many organisms, while others are used in the treatment of some diseases.

Consequently a technique was found in which plant leaves are used in the process of assembling metals (Iravani 2011), where the leaves of the plants are converted into extracts, which in turn convert the metal ions into neutral atoms clustered on the extract molecules. Plant leaves of various kinds possess a considerable number of biochemical compounds that carry functional groups acting as antioxidants because they are rich in non-double electrons (Tilman et al. 1997). It is one of the most recently used methods in the process of synthesizing metallic nanoparticles. *Murraya koenigii* leaf is believed to have several medicinal properties such as antimicrobial, anti-diabetic, hepato-protective, anti-inflammatory, antioxidant, and anti-carcinogenic properties (Al Harbi et al. 2016). The search for suitable ways to produce metal nanoparticles has become a matter of interest recently because the use of metallic nanoparticles has shown high efficiency in many applications. Metallic nanoparticles have a lot of antibacterial properties due to their high surface and small particle size, which gives them an increased surface reaction. Their optical and electronic properties are highly dependent on their structure and size; it is essential to modify the size and composition of metal particles for use in different applications. It is used as an antibacterial, as nanoparticles damage the cellular wall of bacteria (Sirelkhatim et al. 2015). Silver nanoparticles are one of the most recently studied nanomaterials, they play a significant role in the development of the antibacterial activity, as they can get adsorbed on the surfaces of bacterial cell membranes (Le Ouay and Stellacci 2015). Modification of metallic nanoparticles order to obtain many useful properties such as complex structures, purity, stable composition, and several other purposes including reducing the size to nanometer size has been reported (Scaramuzza et al. 2016). The change in sizes and structures produces a different electrical, optical, physical, chemical, and a strong antibacterial property. In this Chapter, the green-synthesized nanoparticles and their medical applications is reviewed. Moreover, the applicability of the metallic nanoparticles (MNPs) in the inactivation of microbial cells was also discussed.

3.2 Nanotechnology

Nanotechnology is a branch of modern technology which is currently useful in manufacturing and describing metal and non-metallic materials on the scale of a nanometer (1–100 nm) in various forms, structures, and sizes (Lengke et al. 2006). There are many products in markets based on nanomaterials, which play an essential role in everyday life. Nanoparticles have unique properties that are different from macroscale properties of the same material, so research interest has increased. Nanomaterials are classified according to origin, dimension, and structure. Depending on the dimension, the materials that take the critical range (1–100) nm in all their three spatial directions are referred to as dimension-zero particles (0-D), such as quantum dot and metal nanoparticles; all these particles are spherical, cubic, or clustered in shape. The particles that have one dimension beyond the critical range and the other two dimensions are within the nanometer range are one-dimensional molecules (1-D), for example nanowire, Nano rod. While molecules that have two dimensions out of the nanometer range and one direction within the range are two-dimensional molecules (2-D), meaning they grow in the two spatial directions; this includes Nano films and Nano sheets. For the particles that have three out-of-nanometer directions, they include bulk materials (Buzea and Pacheco 2017). The nanostructure might be Liposomes, Dendrimers, Carbon-based and metal-based nanostructure. Liposomes are molecules composed of two vesicular layers of natural and industrial phospholipids (Perez et al. 2016). Dendrimers are polymers that are in the form nanoparticles. They are nanomaterials that are based on Carbon, such as carbon nanotube and Fullerene. The metal-based nanomaterials, as the name suggests, are nanomaterials that can be nanoparticles made up of metal that may consist of two, three, or more types of metals or elements (Mazhar et al. 2017).

3.2.1 Nanoparticles

Nanoparticles are engineered at the level of atoms and molecules, giving them unique and superlative properties that are not found in the Bulk Materials. Nanoparticles are considered to be a complete unit concerning their properties. All materials have a critical range in which their properties change. Materials, less than 100 nm, show properties that differ from the properties of the bulk materials. When all particle dimensions are within a range of less than 100 nm, it is known as nanoparticles, such as silica nanoparticles. In the theory of previous studies, nanoparticles are often called nanoclusters because of the accumulation of millions of atoms and molecules (Kunwar et al. 2014). Nanoparticles may be crystals or amorphous as their surface can be translate of drugs by packaging or its association with the surface of nanoparticles. The properties of nanoparticles differ from the properties of atoms, molecules, and bulk materials. Nanoparticles are a unique state of matter, such as crystalline nanoparticles, fullerene, and carbon nanotubes, as were traditional crystalline materials (diamonds and graphite). Researchers limit the size of nanoparticles between 10 and 100 nm. The production of nanoparticles is carried out in two ways: from top-down and bottom-up, from top-down where the bulk materials are broken down into small volumes at the nanoscale; while in bottom-up, atoms and molecules are assembled to obtain nanostructured materials. The use of top-down approaches in the production of nanoparticles has a very high probability of contamination, so a bottom-up approach is appropriate for producing nanoparticles. Today, nanoparticles have become very important in our lives. For example, zinc oxide nanoparticles are one of the products that are included in antimicrobial agents. Titanium oxide nanoparticles are employed in the formation of harmful sun protection products. The basis of nanotechnology is based on the formation of nanoparticles where these nanoparticles are used in different formats depending on the type of applications.

3.2.2 Metal Nanoparticles (MNPs)

Cluster metal particles studied for decades are intermediaries between atoms and bulk materials, and currently involved in all nanoscience applications. Metal nanoparticles are atoms clustered together in a cluster, ranging in diameter from 1 to 100 nm (Chaloupka et al. 2010). Metallic nanoparticles can be involved in many applications such as water purification, catalysis, medical diagnosis, drug delivery, tissue engineering, and antibacterial (Betancourt-Galindo et al. 2014). There are also other areas which include electronics, paint, cosmetics, and packaging. Metallic nanoparticles can be used in solid forms by integrating them when their temperature is relatively low, and they can often be used as electronics coatings without melting. The permeability properties of metallic nanoparticles make them useful in many applications such as coating and packaging. The metal nanoparticles can bind to the DNA chain without destroying it, and have the ability to pass through the blood vessels and target specific organs in the body and thus, opens the way in new medical, diagnostic, and therapeutic applications (Klein et al. 2013). Some metal nanoparticles can decompose simple halocarbons, which pose a risk in carbon and metal halides at room temperature. Metallic nanoparticles are introduced into biomedical applications such as antibacterial functions, bandages, and medical instrument manufacturing. It is known that metal nanoparticles have an antibacterial property due to their high surface area, where extensive interactions happen on the surface of microorganisms, which works to inhibit their job or lead to the death of cells (Slavin et al. 2017). Metal nanoparticles surface plasmon absorption and surface plasmon light dissipating can be utilized for analytic and restorative applications. The chemical and physical properties of nanoparticles and anticancer are also used to deliver drugs to target members (Wu and Huang 2017).

3.2.2.1 Silver Nanoparticles (Ag NPs)

Silver is a transitional element that has different properties such as electric and thermal conductivity and has a metallic lustre. In the past, silver has many benefits, it was used as a remedy, in the manufacture of coins, pots, ointments, and many more (Biswas and Dey 2015). One of the most widely used materials in nanotechnology is silver. Antibacterial agents are increasingly desirable at the nanotechnology level. Silver nanoparticles have wide applications in various fields, including water purifiers, surgical instruments, textiles, and cosmetics (Thamilselvi and Radha 2017). Silver nanoparticles replaced silver sulfadiazine in the treatment of wounds, as they are used as a covering agent in some household appliances and medical surfaces of devices to reduce hospital-related bandages and infections. A silver nanoparticle is prevalent in many health products because of its unique ability to combat infectious diseases and inhibit the growth of bacteria and germs. The important application of silver nanoparticles is in the medical manufacture such as topical ointments to stop the infection from burns and open wounds. Nano-silver particles have much therapeutic utilizations. It has a surface Plasmon resonance and an extensive cross-scattering section, making it ideal for use in molecular adhesives. Biosynthesis of inorganic materials has been reported by many researchers, and it is recognized that these methods are considered clean, inexpensive, and non-toxic to humans and the environment. Interest in research and development has grown around silver nanoparticles due to its unique properties that can be applied to bio-nanotechnology (Fayaz et al. 2011).

3.3 Synthesis Methods of Metallic Nanoparticles

There are two techniques used in the process of synthesizing nanoparticles, bottom-up and top-down (Biswas et al. 2012). A bottom-up approach is a self-generated nanomaterial engineering process where atoms, molecules, or clusters assemble to form small-scale materials on a nanometer. The atoms and molecules are automatically generated in a process called self-assembly under certain conditions, but this approach can consume much time. While in the Top-down approach, the process begins with the use of bulk materials in multiple ways to obtain the smaller sizes commonly used in the manufacture of microprocessors. In recent years, there has been an increase in interest in the production of metallic nanoparticles, because of its unique properties. In many applications, metallic nanoparticles are different metals, each with a distinct function. There are several studies aimed at producing metallic nanoparticles, and the most common methods of producing metallic nanoparticles are the physical and chemical methods.

The physical method uses different processes for the synthesis of metal nanoparticles such as laser ablation, laser evaporation and radiofrequency plasma (Owens and Poole 2008), epitaxy of sub-atomic shaft (Atkinson et al. 2006), and deposition of Physical vapour. In Radio Frequency plasma technique, high voltage is being utilized to produce nanoparticles (Gunnarsson 2018). The method of evaporation-intensification includes the use of an oven at atmospheric pressure, where the required materials are stored inside the boat located in the centre of the oven and evaporated in the gas carrier (Kruis et al. 2000). The method of laser evaporation is where the high-density package is placed on the surface of a metal plate to evaporate the atoms of the metal and then cooled to obtain the nanoparticles using helium gas in the vacuum (Turkki 1999). Physical methods have some major drawbacks such as great energy consumption and take period to obtain thermic constancy (El-Nour et al. 2010).

Chemical methods are widely utilized methods for producing nanoparticles, for example sol-gel and hydrolysis (Danks et al. 2016) and chemical vapour deposition (Palgrave and Parkin 2008). Chemical vapour precipitation includes deposition on an interaction material using vapour of phase precursors as chemical reaction (Khanna 2016). Chemical vapour precipitation has a disadvantage of gases of precursor been liable to fire danger and corrosion (Lukaszkowicz and Dobrzański 2008).

Given restriction of chemical and physical processes, researchers have paid keen attention to improve a secure, eco-friendly, and inexpensive alternate process for the production of nanoparticles (Prakash et al. 2013). The synthesis of nanoparticles by microorganisms and plants is right away widely preferred as a green synthesis eco-friendly and inexpensive approach to the synthesis of nanoparticles of accurate forms and controlling temples (Gurunathan et al. 2009). Various bio-systems like fungus (Gholami-Shabani et al. 2013), bacteria (Kumar and Mamidyala 2011), plant extracts (Shankar et al. 2004), and yeast (Kowshik et al. 2002) have been applied in nanoparticles synthesis.

3.4 Bio-nanotechnology

Scientists often rely on inspiration from nature for their research (Marrow 2000; Cossins 2015). Large concentrations of nanoparticles have been detected by organic processes (Haferburg and Kothe 2007). Several subsequent studies have shown that many plant extracts containing biomolecules can produce inorganic nanoparticles that accumulate on the surface of biomolecules. Therefore, there has been tremendous development in area of nanoparticles synthesized by plants. It is clear that there is increasing interest by scientists on inorganic particles reaction with bio-species through the backlog of many studies on producing diverse of NPs by biochemistry. The groups contain NPs such as silver, gold, and alloys (Sharma et al. 2016). Bio-nanotechnology is an emerging technology with great interest in the use of green synthesis in material production (Kumar et al. 2016).

Bio-nanotechnology is known to be used as a building block and to take advantage of bio-activity to develop modern nanotechnology (Ehud and Anna 2013). Biotechnology combines vital biological essentials to chemical and physical methods in the synthesis of nanoparticles. It is as well the economical alternative to physical and chemical processes in the formation of nanoparticles (Patil et al. 2012). The use of chemical and physical methods to produce nanoparticles is very limited in bio-applications, especially in the field of medicine. The use of biological systems as an alternative to chemical and physical methods is appropriate and environmentally friendly. Thus, the use of safe, non-toxic, environmentally friendly alternative methods to develop nanoparticles synthesis is of most importance for the expansion of medical applications. Compared with biomolecules, chemicals are considered expensive and are replaced by a green method that is more acceptable and energy-consuming than chemicals and environmentally friendly. The biological approach supports more, as this process occurs at standard conditions of varying temperatures, pH, and pressure, the nanoparticles resulting from this process are more catalytic reactive and have a high surface area (Bhattacharya and Mukherjee 2008).

Synthesis of nanoparticles by a biological method is a isolate of bottom-up approach, wherever the primary interaction in this process is reducing and capping metal ions to complete nanoparticles synthesis (Nalawade et al. 2014). NPs are synthesized when biomolecules grabbing purpose ions of their medium then turnover the ions to the atoms over functional groups present. The metal ions are reduced and converted into neutral metals aggregated on the surface of the biological molecules because of the presence of functional groups rich in free electrons (Carter 1995). The NPs are used in various applications such as medicine, DNA analysis, cancer treatment, antibacterial agents, and biological sensors. Currently, its applications have been explored in many advanced technological fields (Punjabi et al. 2015).

Various plants, proteins, and microscopic organisms have been used to produce metal NPs (Ahmed et al. 2016). There are several reports on the production of silver nanoparticles by biotics such as bacteria (Saifuddin et al. 2009), yeast (Pimprikar et al. 2009), fungi (Rai et al. 2016), plants, and plant extracts (Anandalakshmi et al. 2016), there may be much work on the production of nanoparticles silver due to the importance of utilize for many years. Several works have as well been studied on the production of metal nanoparticles with plant extracts, and it described the use of plants in the production of nanoparticles to be safe, economic, environmentally friendly, and efficient in production (Mittal et al. 2013). There are some reports on the production of metal nanoparticles with plants.

The synthesis of nanoparticles using the plant system provides alternative and cost-effective methods in a wide range of biomedical applications (Parveen et al. 2016). However, there are many reports which reveal that synthesis of nanoparticles using plant extracts requires different temperatures (Song and Kim 2009), or the use of microwave radiation (Hebbalalu et al. 2013), because the formation of metal nanoparticles using these kinds of plants is very slow. A significant advantage in Table 3.1 is that a lot of this synthesis is present in silver nanoparticles though the

Table 3.1 Metal NPs green synthesis with some plants

Plant leaf extract	Metal or Metal oxide NPs	Shape of nanoparticle	References
Leptadenia reticulata	Ag	Spherical	Swamy et al. (2015)
Melissa officinalis	Ag	Spherical	Reyes-López et al. (2017)
Alternanthera dentate	Ag	Spherical	Kumar et al. (2014)
Moringa oleifera	ZnO	Hexagonal	Elumalai et al. (2015)
Centella asiatica	Ag	Spherical	Rout et al. (2013)
Ziziphora tenuior	Ag	Spherical	Sadeghi and Gholamhoseinpoor (2015)
Rosmarinus officinalis	Ag	Spherical	Ghaedi et al. (2015)
Croton sparsiflorus morong	Ag	Cubic	Kathiravan et al. (2015)
Catharanthus roseus	Pd	Spherical	Kalaiselvi et al. (2015)
Hippophae rhamnoidesLinn	Pd	Spherical	Nasrollahzadeh et al. (2015)
Hibiscus subdariffa	ZnO	Spherical	Bala et al. (2015)

production of other metal nanoparticles such as Au and Pd has also been studied. Besides, it is monitored a lot of biosynthesis of Metal NPs with plant are spherical, though triangular, hexagonal have been observed (Dubey et al. 2010). And shows that silver is the most widely discovered minerals with plant and that the synthesized NPs are essentially spherical. They argue that the dominance of Ag NPs may be because of the huge applications it has in the area of biomedical applications (Ahmed et al. 2016).

3.4.1 Murraya Koenigii Plant

Murraya koenigii, usually known as curry plant or in Indian dialects called *karipatta*, belongs to the Rutaceae family representing more than 150 gender and 1600 species (Satyavati et al. 1987). *Murraya koenigii* is a valuable plant for its distinctive aroma and medicinal importance. It is an important export commodity from India because it brings good foreign revenues. The number of chemical components of each part of the plant has been extracted. Some of the important chemical constituents such as P-elemene, O-flandrin, P-caryophylene, and Pgurjunene are responsible for the characteristic odour. The plant is considered a rich Carbazole alkaloids source (Kumar et al. 1999). The Bio-active Acridine, Coumarin alkaloids, and Carbazole alkaloids were studied from the Rotasia family by Ito'o (Ito 2000). *Murraya koenigii* is most utilized in cooking for centuries and has a versatile role in traditional medicine. The leaves, roots, and barks of the plant are utilized as externally, anthelmintic inflammation and stimulant to treat bites and eruptions of toxic animals. Raw green leaves are eaten to treat vomiting, dysentery, and diarrhoea.

The *Murraya koenigii* or Curry is a small tree with leaves called Curry leaves, it is native to India, Sri Lanka and is always found in tropical and subtropical areas. Different countries such as Nigeria, China, Ceylon, and Australia cultivate Curry tree. The plant height ranges from small to medium, growing (4–6) m high, with about 40 cm trunk, having 16 inches diameter. Leaves, barks, and roots are the useful parts of this plant. In India and Sri Lanka, cooking leaves are valued as a seasoning, fried with vegetable oil. The leaves are highly appealing for some health benefits, depending on the intended use, they can be fried or dried. The fresh curry leaf is very popular for herbal medicines. Curry leaves are believed to have several medicinal properties which include antimicrobial, anti-diabetic, hepato-protective, anti-inflammatory, and antioxidant properties. The plant contains several pharmacological activities, which acts on cholesterol-reducing property and cytotoxic activity. It is known that this plant is considered the richest source of Carbazole alkaloids (Meena et al. 2017).

3.4.1.1 Chemical Leaves Contents (Phytochemicals)

Murraya koenigii includes many chemical components that interact in a combined way to stimulate their pharmacological response. Some isolates active ingredients responsible for medicinal properties have been characterized. This plant has an antibacterial, antioxidant, cytotoxic, anti-ulcer, and cholesterol activities and has been reported (Srinivasan 2005). *Murraya koenigii* is a more or less aromatic shrub or a small tree cultivated for its aromatic leaves. In the traditional medicine system, it is utilized as anti-diarrheal, dysentery, blood purifier, tonic factor. The major oil of the leaves yielded D-sabinene, Di-alpha phellandrene, D-terpinol, and Caryophyllene (Gopalan et al. 1980). It is reported to have antimicrobial, antioxidant, antifungal, anticancer, hypoglycaemic, anti-fat, and pro-active effects against liver damage caused by carbon chloride in mice (Parimi et al. 2014). Also included are Cystosterol and Carbazole (Gupta et al. 2009). The chemical composition of fresh leaves from *Murraya koenigii* consists of variable oil. Curry leaves are rich in phosphorous, calcium, magnesium, iron, copper, vitamin A, vitamin B complex, vitamin C, vitamin E, antioxidants, folic acids, a plant sterol, amino acids, carbohydrates, glycosides, fibres, and flavonoids.

3.4.1.2 Anti-oxidative Property

Carbazole alkaloids isolated from the dichloromethane extract of *M. koenigii* leaves were evaluated based on the oil stability index along with the radical DPPH scavenging ability based on the slow time to reach a stable state. The carbazole ring has an aryl hydroxyl substituent on it, and it is suggested to play a role of reduction and stabilizing against free radicals (Tachibana et al. 2001). The antioxidant properties of *Murraya Koenigii* extracts were evaluated using different solvents based on the OSI oil stability index with a radical scavenging capacity against 1, 1-diphenyl-2-picrylhydrazyl (Kureel et al. 1969). Koenigine and Mahanimbine, two alkaloids of carbazole, isolated from *Murraya koenigii* leaves showed antioxidant activity. *Murraya koenigii* also showed a high degree of properties of radical scavenging (Borse et al. 2007). Curry tree containing many chemical compounds, cinnamaldehyde found in, barks, seeds, and stems, and numerous alkaloids of carbazole, is an organic compound has formula $C_6H_5CH{=}CHCHO$. Carbazole is one of the organic compounds, and it has an aromatic heterocyclic and a tricyclic structure, including two rings of benzene fused on either side ring of five-membered nitrogen.

3.4.1.3 Antimicrobial Activity

The known carbazole alkaloids were isolated from the stem bark of *Murraya koenigii*. These compounds were found to be effective in the 3.13-concentration range of 100 μg/mL (Rahman and Gray 2005). The literature study revealed that extracts from different plant species were examined in an antibacterial activity in the laboratory against multiple bacterial resistance isolates, including Gram-positive and negative strains. The study showed that *Murraya koenigii* has shown antibacterial activity (Panghal et al. 2011), another study used C*urry* leaves to investigate the antibacterial effects; the leaves were powdered, and antimicrobial activity on Gram-positive and Gram-negative bacteria was tested, the curry leaf extract showed inhibition zone against bacteria culture.

3.4.1.4 Leaves Extracts and Bio-reduction

Plant extracts are mightily utilized in applications of the industry, and the possibility to effect development of bioscience. Plant is the essential producer of the aqueous system (Ingale and Chaudhari 2013), and its role in preventing the oxidation of metals and molecules from the environment often leads to the removal of toxicity in the environment through degradation. It is said that bio-sources collect metals through two steps, operation of biodiversity. This part is executed using functional groups of vital compounds contained (Abdi and Kazemi 2015). In 2013, Bindhu and his colleagues used the extract of *Hibiscus cannabinus* leaves with the concentration of various metal ions and the reduction of ions and their conversion to neutral metal nanoparticles at different reaction times (Bindhu and Umadevi 2013). A similar study conducted by Ravichandran, et al. reported the synthesis of nanoparticles using plant leaf extract to reduce Ag^+ ions to Ag nanoparticles from $AgNO_3$ solution during two minutes of reaction time at room temperature (Ravichandran et al. 2016). *Murraya koenigii* leaves extract is used for the synthesis of nanoparticles, and this synthesis is attributed to the natural reduction agent, the carbazole may be present in the corresponding extracts (Kapoor and Singh 2010). Curry leaves have a potent antioxidant due to the presence of high concentrations of carbazoles that is responsible for the reducing and stabilizing of metal ions.

3.4.2 *Green Synthesis of Metallic Nanoparticles with* Murraya Koenigii

Different methods have been used with plant extracts to synthesize metal nanoparticles. These methods have many features over physical, chemical, and microbial syntheses because there is no need of the process of elaborated for maintaining and culturing the cell, hazardous substances, and high-energy requirements (Awwad et al. 2013). The synthesis of metallic nanoparticles with plant leaves is further bio convenient comparing with other biological processes. It attracts much attention from researchers to the extent that area of research on the utilize of plant leaf extracts in nanoparticles produce called photosynthesis, become an eminent area of study in nanotechnology (Ahmed and Ikram 2015). However, gold and silver nanoparticles were synthesized rapidly using curry leaf extract (Philip et al. 2011). Curry leaves

Table 3.2 The related works on the synthesis of MNPs by *Murraya koenigii* leaf

Extract used	Precursor	Concentration (mM)	NP of element	Researcher
Curry leaves	$AgNO_3$	1	Ag	Christensen et al. (2011)
Curry leaves	$AgNO_3$, $HAuCl_4 \cdot 3H_2O$	1	Ag, Au	Philip et al. (2011)
Curry leaves	$AgNO_3$	1	Ag	Bonde et al. (2012)
Curry leaves	$AgNO_3$	1	Ag	Vivekanandhan et al. (2012)
Curry leaves	$AgNO_3$	1	Ag	Suganya et al. (2013)
Curry leaves	$AgNO_3$	1	Ag	Deb (2014)
Curry leaves	$AgNO_3$	1	Ag	Sajeshkumar et al. (2015)
Curry leaves	$AgNO_3$	1	Ag	Kamaraj et al. (2017)
Curry leaves	$CuSO_4 \cdot 5H_2O$	1	Cu	Ashtaputrey et al. (2017)

have been used in traditional medication as a therapy for various diseases. To extend the reduction mechanism of *Murraya koenigii* leaf, need for detailed investigation is necessary in order for further applications. Fresh Curry leaves that diverse researchers have studied, have the ability to synthesize metallic nanoparticles. Curry leaf extraction is found in the synthesis of nanoparticles used. The related work is listed on the synthesis of metallic nanoparticles (MNPs) with curry leaves extract (Table 3.2).

The bio-molecular synthesis uses plant extracts as a result of the existence of active biomolecules present in the plant cell (Sorbiun et al. 2018). Kamaraj et al. (2017) monitored that when the extract of original curry leaves was treated with Ag NO_3, a large proportion of precursors were taken with the resulting formation of silver NPs through one day. This rapid ratio of accumulation is following by the formation of metal nanoparticles that refer to change in mixture colour to yellow which was colourless. Silver ions are trapped on the surface of the biomolecules by the electrostatic reaction, and the ions are then reduced by functional groups. The interaction of these biomolecules with metal ions makes a reduction in many metallic ions leading to the aggregation of nucleus into metallic nanoparticles (Shah et al. 2015). Metabolites of plant such as proteins, sugars, polyphenols, terpenoids, phenolic acids, and alkaloids play a significant role in reducing metal ions to nanoparticles and supporting their stability (Shankar et al. 2003). The increased efficiency of biochemical immediate by the plant extract can mediate the biosynthesis of silver NPs. In addition, the components of amino acids in the protein residues have the capacity to link with metals. This proposes the forming of a surround coat on metallic NPs that as well act as stabilization to prevent aggregation and provide high stability metallic nanoparticles.

These monitoring confirm the potential that lipidemic, glycemic, antioxidant, free radical scavenging, and antimicrobial activities act to reduce and as well as stability agents in silver nanoparticles (Ningappa et al. 2008). Thus, antioxidants tocopherol, carotene, and lutein are vital in the synthesis and stabilization of silver nanoparticles (Devatkal et al. 2012). Silver NPs are synthesized on the biomolecules surface without any conglomeration. In addition to the initial visual monitoring of colour change, their other characteristics have led to more characteristics of the biosynthesized silver NPs. The UV-visible spectra exhibit a sharp band of SPR at the peak of 420 nm, indicating that spherical and relatively small silver NPs.

According to Ovais et al. (2017), the reduction of Ag ions to Ag atoms occurred with the involvement of phytochemicals found in the plant leaves extract, nanoparticles released found with these biochemical compounds. Recently, Swamy et al. (2015) claim that functional groups on the surfaces of biochemist's work on reducing ions. The mechanism indicates that different biochemical groups that existed to be associated with plant extract have function in reducing ions into nanoparticles. Ahmad et al. (2010) suggest that different roles are played using various compounds or molecules of diverse plants in the synthesis of metallic nanoparticles and proposed a generic mechanism for synthesis of metal nanoparticles by plant extract.

3.4.3 Modification of Nanoparticles Using Laser Irradiation

The expectations of the technical revolution are related to nanotechnology, but the formation, modification, and use of objects of smaller dimensions in research and science are gaining so much credence (Köhler and Fritzsche 2008). The functional properties of nanoparticles make them more ideal as different blocks to build structures of high dimensions of technological significance. In this material, particles act also on known molecules and are organized into thin films, monolayer, and super lattices for nanoscales. The change in the molecular length makes it potential to include quantum transformations and transfers from non-conductor to conductor, leading to compressive electronic and photovoltaic properties (Wang 2000).

Laser ablation can be used for a solid purpose in a liquid medium to manufacture nanostructures with different compositions (e.g. metals, metal oxides, alloys, hydroxides) as well as various morphological forms, for example nanotubes, nanorods, and nanocomposites. The manufacture of nanostructures using laser radiation in liquids is easy and green process that can be executed in normal conditions in organic or water fluids. Laser ablation in liquid has prepared a series of nanoscale materials with morphologies, microscopic structures, and distinctive phases in order to search for new properties (Zeng et al. 2012). Nanomaterials have been manipulated by laser irradiation extensively in the past using different methods used for melting, fragmenting, or reshaping nanoparticles. It is reported that laser beam radiation either results in particle fragmentation or fusion due to the melting of photovoltaic heat (Zamiri et al. 2011). This can be considered an opportunity to use lasers to control the shape and size of nanoparticles. Additionally, there is a possibility to manufacture nanotubes and nanowires by ablating solid materials in laser liquid media. Recently, researchers benefit from laser ablation in liquid appropriately because of the unique properties of nanotechnology with new and changing morphologies (Lin et al. 2010).

Formation of nanoparticles using laser ablation at surface plasmon resonance frequencies can supply an efficient means of changing sizes and shapes of NPs. That is specially, so because the sizes of the particles, along with the surface-absorbing species and the insulating medium, influence the situation and form plasmon absorption. The application of laser ablation on a huge numeral of materials is possible. It is easy and eco-friendly for producing nanoparticles with a trend to synthesis distributions of relatively large size (Burakov et al. 2005). There are frequent reports on using Nd:YAG laser to synthesize metal nanoparticles. This laser Nd:YAG (Neodymium doped Yttrium Aluminium Garnet) has a short length. That is convenient for pulse applications requiring more profound breakthrough, covering smaller areas as well as precise processing for specified purposes. Laser Nd-YAG is a practical option for procedure involving various modifications occurring in a short period. In such procedure, the laser energy as well as reaction period is decisive (Majumdar and Manna 2003). Pules laser ablation in liquid is a promising, multilateral, easy, and technical technology with a broad zone of attainable materials. Laser ablation has been used in the production of varied nanoscale structures. Pulse laser ablation in liquid of NPs results in the formation of self-regulating nanostructure materials which sizes are monitored based on the pulse period (Barmina et al. 2010). Modification of nanoparticles by laser irradiation includes nanorods, nanocubes structures and related nanostructures, arranged arrays of NPs, and heterogeneous structures (Kim et al. 2017).

3.4.4 Reshaping and Fragmentation of Metal NPs Using Laser Irradiation

The modification of Ag NPs shape with laser pulse involves the interaction of laser radiation in resonance with nanoparticles for transformations to plasmonic nanocrystals. The first investigations of the effect of laser pulses on the colloidal dispersions of spherical NPs showed that reshaping and fragmentation were the significant influences caused by the intensity of the laser pulse (Kurita et al. 1998). In 2000, Link. et al. used laser pulses at high influence for fragmentation and reshaping of nanomaterials into sphere nanoparticles (Link et al. 2000). The fragmentation and reshaping phenomena are dominated by the electron dynamics of the nanomaterials and the relaxation procedure after laser irradiation, photons are absorbed when laser excitation is subjected, producing fast thermal electrons, followed by energy of electron lattice transfer and that heat transport to the surrounding (Ahmadi et al. 1996; Logunov et al. 1997).

Laser irradiation at nanosecond pulse experiments, the absorption of photons continues, when the relaxation procedures start and still lattice hot, yielding an increase in lattice energy that produces sequent fragmentation of the nanomaterials (Link et al. 1999). However, not only pulse width has effects related but also the pulse energy, high-energy short pulses, and low-energy long pulses also can produce melting and fragmentation of nanomaterials. When the nanostructures are irradiated by a short laser pulse, the electromagnetic field is magnified at the point where a sudden change occurs in shape. This leads to a sudden concentration of energy at that stage, leading to fragmentation of particle (Ueno et al. 2008). Evaporation, melting, or both occur on the surface of the particle due to temperature imbalance between the surface of the particle and the internal, which is likely to fragment the particles.

In contrast to the theory of photothermal evaporation, this Colombian explosion model indicates that electron thermal emission occurs with the relaxation of the electron-photon (Yamada et al. 2007; Werner et al. 2011). NPs surface melt when the lattice temperature reaches 700 K, which gives rise to reshape phenomena, while increase in structure temperature up to 1337 K will melt metal NPs. Accompanied temperature increase thermionic emission of the electrons, which might go up to the critical state of the colloid nanoparticle, which in turnover becomes unstilled and breaks up into smaller droplets (Pyatenko et al. 2013). The laser-induced size decrease has attracted the necessary attention from many researchers in the associated fields. This phenomenon contains the essential aspects of the interaction between nanoparticles and laser irradiation such as how photon energy flowing within nanoparticles results in a devastating event. Besides, the method might be used to control particle and distribution size in laser-pulse-based nanoparticles generation (Semaltianos 2010).

3.4.5 Fabrication of Colloid Nanostructures Using Laser Irradiation of NPs

Modification of the optical properties through the reshaping of optical structures is possible. Further, the production of high monodisperse metal nanostructures has been reported by the reshaping of colloidal metal nanoparticles utilizing laser irradiation at 532 nm nanosecond (Bueno-Alejo et al. 2012). The combination of laser pulses with metal NPs is still under emergence, it is vital to utilize in chemical and biochemical processes. For example, the wide area of high temperatures reached by excitation of metallic nanoparticles with short laser pulses might lead to new advances in practical catalysis and organic chemistry (González-Rubio et al. 2016). Synthesis of metal NPs with high purity, small size, and narrow size distribution is essential in the fields of medicine and electronics applications. Silver nanoparticles have antibacterial properties; it is considered to be a powerful contender in the critical study for a response to antibiotic-resistant bacteria. Researchers have used pulsed laser irradiation to discover the bacterial effect. This is promised development in the combat against antibacterial resistance.

Recently, continuous wave diode laser (CWDL) has more attention which used in many applications. There is a close link between diode laser beam and metal nanoparticles. Metal nanoparticles respond to laser light. Nanoparticles of different shapes and sizes were analyzed via laser diode (Beckmann et al. 2012). The detection of nanoparticle variability is illustrated by thermal light modification (Adler et al. 2008). CWDL is used to release an encapsulated polyelectrolyte capsules based on laser light irradiation with metal nanoparticle (Skirtach et al. 2004). Similarly, the absorption ability of metal nanoparticles by photothermal is used in closing wounding (Matteini et al. 2012).

Diode laser irradiation (DLI) has been developed in photothermal therapy by profiting on the absorption of metal nanoparticles in the blue range (Kelkar et al. 2016). DLI with a 450 nm light source has a presumed fungicidal impact on *C. albicans* biofilm through irradiated silver NPs and provides a reduction of biofilm. Furthermore, silver nanoparticles remediated with a different exposure time of diode laser irradiation stimulated and produced antifungal impact (Astuti et al. 2017). Irradiation of continuous wave (CW) in conjunction with nanoparticles to realize visible emission and the capacity to sensor and lighting in complex biological places was reported (Fernandez-Bravo et al. 2018). Another study comparison of the efficiency of laser-activated nanoparticles and traditional photodynamic therapy showed that activated nanoparticles could be used as an adjunct to purify the root canal system (Afkhami et al. 2017).

Kumpulainen et al. (2011) have reported that laser calcification of nanoparticles using continuous wave allows for short sintering and selective sintering which makes it possible to produce nanostructures (Kumpulainen et al. 2011). Experimental probes of the excitation processes of nanoparticles under the excitation of CW were performed on the foundation of the quantum transition (Zhang et al. 2016). Also, effective and selective manipulation of various nanoparticles by blue laser irradiation that is higher in energy through measuring the time of individual partial detention and preventing movement of particles has been reported (Kudo et al. 2017).

3.5 Antimicrobial Activity of Nanoparticles

The application of nanotechnology in the wastewater treatment has been reported in the literature (Athirah et al. 2019). Noman et al. (2019) investigated the inactivation of antibiotic-resistant *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) seeded in greywater by bimetallic bio-nanoparticles. The bimetallic nanoparticles (Zn/Cu NPs) were biosynthesized in secondary metabolite of a novel fungal strain identified as *Aspergillus iizukae* EAN605. The study revealed high efficiency for Zn/Cu NPs in inhibiting the growth of *E. coli* and *S. aureus*. The inactivation mechanism revealed that the bacterial cells were inactivated due to the damage in the cell wall structure as well as the degradation of carbohydrates and amino structures on the bacteria cell wall. The Fourier transform infrared spectroscopy (FTIR) analysis confirmed that the destruction takes place in the C-C bond of the functional groups available in the bacterial cell wall. Moustafa (2017) investigated the inactivation of pathogenic bacteria including *E. coli*, *Staphylococcus* sp., and *Pseudomonas* sp. in the water samples by using biosynthesized Ag NPs in the extracellular metabolic of *Penciillium citreonigum Dierck* and *Scopulaniopsos brumptii Salvanet-Duval*. The antibacterial activity of biosynthesized Ag NPs exhibited the inactivation based on the interaction with bacteria. Baek et al. (2019) studied the efficiency of ZnO nanoparticles (NPs) in an alginate biopolymer solution to inactivate antibiotic-resistant bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) in water. The results revealed that both bacteria are removed by 98% and 88%, respectively. The maximum removal was recorded with a high dose of NPs (4, 10, and 20 mg). These findings concluded that ZnO NP–alginate beads have high applicability in the water treatment. Bhattacharya et al. (2019) investigated the applicability of CuO NPs in treating wastewater. Moreover, the effect of pH, stirring speed, concentration of algal extract and Cu^{2+} was optimized. CuO NPs exhibited antimicrobial activity against *Escherichia coli* with 50 μg/mL. The toxicity assay on fish model revealed that CuO NPs have no micronuclei for 30 days. The study claimed that the green-synthesized CuO NPs in drinking water applications.

3.6 Conclusion

It can be concluded that green synthesis technology for bio-nanomaterials has several advantages in comparison to the chemical and physical methods. Moreover, the applicability of these materials to inactivate infectious agents in the water and wastewater might be high due to the antimicrobial activity of the plant extract.

Acknowledgements The authors would like to thank Ministry of Education Malaysia for providing FRGS vot K171 (Investigation Factors Determining Zwitter Characteristic Phenomena In Polymer Resist Materials For Semiconductor Industry Application) as a financial support for this research project.

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Chapter 4
Treatment Technologies of Fresh Market Wastewater

N. M. Jais, M. S. Miswan, Radin Maya Saphira Radin Mohamed, and Adel Ali Saeed Al-Gheethi

Abstract Fresh Market Wastewater (FMWW) is rich with high amounts of suspended solids, organic and inorganic compounds, nutrients, gases and some elements which come from fish entrails and seafood preparation sales, meat cutting, poultry slaughtering, fruits and vegetables sales. The excess of these parameters are harmful to the aquatic life since the FMWW is usually discharged into the drainage systems without any treatment or partial treatment. The FMWW technologies are developed due to some technical factors influencing the designation and construction. There are several projects of fresh market treatment technologies used on site area in small scale which could be replicated to other fresh market, and some case study have been tested in laboratory batch experiments. All the projects exhibited an efficiency to reduce critical parameters in FMWW and give positive impacts to the locals and responsible parties.

Keywords Fresh market · Wastewater · Treatment technologies

4.1 Introduction

The origin of fresh market is rooted in periodic marketing (typically for a few hours in the morning once every three to five days). Fresh market is the public market for locals to get their fresh foodstuffs such as vegetables, fruits, fish, seafood, chicken and meat. Fresh market has regular customers and is well known as a supplier of good quality raw food. The sellers did not use frozen method for their foodstuff. The customers can bargain with the sellers and compare with the supermarket which has fixed price and usually, the price at fresh market is still affordable (Sze-ki 2008).

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A. A. S. Al-Gheethi et al. (eds.), *Prospects of Fresh Market Wastes Management in Developing Countries*, Water Science and Technology Library 92,
https://doi.org/10.1007/978-3-030-42641-5_4

Unfortunately, the fresh market wastewater (FMWW) generated from fish entrails and seafood preparation sales, meat cutting, poultry slaughtering, fruits and vegetables sales affects negatively on the aquatic life and human health due to the improper disposal into the environment (Zulkifli et al. 2012; Godos et al. 2012). The removal of organic compounds and nutrients is the major requirement during the treatment process of FMWW to meet the acceptable limit before the final discharge to the environment and natural water systems (Jais et al. 2016; Apandi et al. 2019). The current chapter reviewed the advantages and disadvantages of the different FMWW treatment technologies.

4.2 Fresh Market Wastewater Composition and Characteristics

The fresh market wastewater contains suspended solids, organic and inorganic compounds, nutrients, gases and some elements in the wastewater (Apandi et al. 2018). The suspended solids are divided into non-filterable/suspended either settleable or non-settleable and filterable (colloidal or dissolved). The suspended solids in FMWW could increase the turbidity which prevents the light penetration and causes the fish's gills to get plugged up. The silting decreased the depth of river or lake. Moreover, it reduces the lifetime of lakes and blocks the fish to free swim into deeper area. The organic compound such as biodegradable organic which consists of Oil and Grease (O&G), protein increase Biochemical Oxygen Demand (BOD) and blocking oxygen in atmosphere to enter the water (Maizatul et al. 2017). The pollution by fat traps the trash and other materials causing odour, attracting flies and mosquitoes that come out with other disease vectors. The surfactants in fresh market wastewater are refractory organics that come from detergent which had been used for floor cleaning (Zulkifli et al. 2012; Apandi et al. 2017; Jais et al. 2018).

Nutrients are inorganic compounds and are divided into dissolved inorganics (calcium, sodium and sulphate) and nutrients (nitrogen and phosphorus). These compounds are coming from the fresh food itself, the preservatives to maintain the freshness of the fresh food and from water usage. The nutrients are harmful to aquatic life causing the algal blooms and eutrophication. The gases such as nitrogen, oxygen, carbon dioxide and ammonia can be toxic to humans and aquatic life. The elements such as zinc (Zn^{2+}) and iron (Fe^{2+}) from fresh food even in small quantities can be toxic and hazardous to fish and plant in the river or lake where the wastewater flows to the drainage and flows into the river or lake without treatment (Jais et al. 2015, 2016).

The compounds in several FMWW samples investigated in the literature are illustrated in Table 4.1. Zulkifli et al. (2012) and Jais et al. (2015) revealed that the values of Total Suspended Solids (TSS) were between 60 and 900 mg/L which is higher compared to the standard (50–100 mg/L). BOD ranged from 68 to 293 mg/L, while Chemical Oxygen Demand (COD) ranged from 381 to 1440 mg/L which exceeded

Table 4.1 Compounds in fresh market wastewater (Zulkifli et al. 2012; Jais et al. 2015)

Parameter	Ampangan public market	Seremban public market	Seremban Jaya public market	Rasah Jaya public market	Parit Raja public market
TSS (mg/L)	60–122	192–544	250	900	132.3 ± 1.7
Turbidity (NTU)	ND	ND	ND	ND	66.0 ± 8.9
BOD (mg/L)	71–122	137–295	68	153	89 ± 3.61
COD (mg/L)	381–560	656–1440	397	763	456 ± 8.19
O&G	13–43	40–72	28	10	5.22 ± 0.07
TN (mg/L)	30.3–37.3	55.2–72.9	41.6	61.1	36.9 ± 0.5
PO_4 (mg/L)	22.2	2.2–39.6	27.7	44.5	1.61 ± 0.13
Zn (mg/L)	ND	ND	ND	ND	0.312 ± 0.0021
Fe (mg/L)	ND	ND	ND	ND	1.071 ± 0.0010

[a]ND is not determined

the permissible standard (20–50 mg/L and 50–100 mg/L, respectively). Moreover, the O&G is available in the range of 5.2–72 mg/L. The Total Nitrogen (TN) was in the range of 36.9–72.9 mg/L while phosphate (PO_4) was ranged from 1.61 to 44.5 mg/L.

4.3 Technical Factors Influencing Designation of the Treatment System

The factors which need to be considered to design and implement the treatment systems for FMWW include fresh market wastewater content, volume, space area availability, compliance with the environmental standards and regulation on the country as well as compliance with engineering standards for design and construction. The fresh market wastewater usually contains solid wastes and liquid wastes as well as O&G. Therefore, the pretreatment is needed for separating the solids particles and wastewater (Shahbuddin et al. 2018). Fresh market produces huge amount of wastewater quantities since the daily operation of fresh market. Some of fresh markets operating half-day from early morning to afternoon (7 am to 1 pm), while some from early morning to evening (7 am to 6 pm). The estimation of wastewater volume produced per stall per day is 30 litres. The designation of treatment system needs to be sized according to the expected volume of wastewater. The retention time also needs to be considered to ensure the process of wastewater treatment works effectively (Jais et al. 2016). The space area around the fresh market is needed to implement the treatment system. The dimensions of the structure must be suited to the available space (Karia and Christian 2006). Moreover, the effluent or sludge quality must meet the relevant environmental standards before final disposal into the rivers or lakes (Karia and Christian 2006; Jais et al. 2016). On the other hand, the system should be designed and constructed according to the engineering standard, for example the concrete strength and suitable maintenance (Karia and Christian 2006).

4.4 Fresh Wastewater Treatment System

The wastewater treatment system is designed under several stages which are preliminary treatment system, primary treatment system, secondary treatment system and tertiary or advanced treatment system. Preliminary treatment system is a method to remove large inorganic particulate contents of wastewater or floating materials that disturb the operation in primary and secondary treatment of wastewater. The preliminary treatment system includes bar screen for screening large floating materials while grit chamber for heavy suspended solids. Some of the preliminary treatment systems have skimming tank to trap oil and grease which is suitable for fresh market wastewater. The primary treatment system aims for the separation of solids through sedimentation or settling process in sedimentation tank. The wastewaters are left in the tank for several hours to allow the particles to settle at the bottom of the tank. The settleable suspended solids which are called as sludge are then removed or drawn from the tank. This sludge may reduce 60–70 % of fine settleable suspended solids which include about 30–32 % of organic suspended solids (Karia and Christian 2006).

After primary treatment, the biological treatment known as a secondary treatment system is needed to remove colloidal and soluble organic matter present in wastewater. Biological process is employed in this stage. The activated sludge process (an aeration basin with return sludge activity) or trickling filter (a basin with fixed-filter media filter) and secondary settling tank take place in this stage. During the process, the aerobic microorganism needs oxygen to absorb the organic matter from wastewater. Other biological treatments provided for secondary treatment system to cater specific needs are waste stabilization ponds (also known as oxidation ponds), oxidation lagoons (aerated lagoons), oxidation ditches (extended aeration system), Rotating Biological Contractor (RBC), Up-flow Anaerobic Filter (UAF) and Up-flow Anaerobic Sludge Blanket (UASB) (Karia and Christian 2006).

The tertiary or advanced treatment system is needed if the effluent quality of secondary treatment system is unsuitable for final disposal requirement into the rivers or lakes. The tertiary treatment usually focused on the removal of microorganisms, nutrients, elements and gases. The different techniques can be used for tertiary treatment as in Table 4.2.

Table 4.2 Techniques to complete removal or reduction for tertiary treatment system

Purpose	Techniques
To remove residual of suspended solids	1. Granular-media filtration 2. Ultra-filtration 3. Micro-strainers
To remove nitrogen, chlorine and dissolved gases	1. Biological nitrification or denitrification 2. Ion exchange 3. Air stripping
To remove nitrogen and phosphorus	1. Biological process (phycoremediation) 2. Chemical process
To remove dissolved inorganic solids, refractory organics, toxic and complex organic compound	1. Ion exchange 2. Reverse osmosis 3. Electrodialysis 4. Chemical precipitation 5. Adsorption

4.5 Type of Treatment Technologies

The selection of treatment system is depending on the pollution level and level of treatment required to bring the raw wastewater quality to the permissible level of treated wastewater which is safe for disposal or acceptable for specific reuse and recycling. Nowadays, there are three types of treatment technologies suitable for fresh market wastewater which are anaerobic pond, Up-flow Anaerobic Sludge Blanket (UASB) and anaerobic reactor.

4.5.1 Treatment Technologies Implemented on Site Area

There are several treatment technology projects related to fresh market wastewater implemented on site area which are located at

(a) Five fresh markets in Kuala Lumpur, Malaysia (Pasar Harian Selayang, Pasar Borong KL, Pasar Jalan Klang Lama, Pasar Air Panas and Pasar Sentul)
(b) Muntinlupa City Public Market, Muntinlupa City, Metro Manila, Philippines
(c) San Fernando Public Market, San Fernando Cist, La Union, Philippines
(d) Santa Ana Public Market, Manila City, Metro Manila, Philippines.

In Malaysia in 2016, the Department of Civil Engineering and Urban Transportation implemented five wastewater treatments in fresh market in Kuala Lumpur. The sources of wastewater discharge are from wet market activities, discharge from food stalls within the market vicinity and cleaning the market daily after operations. The project locations are Pasar Harian Selayang, Pasar Borong KL, Pasar Jalan Klang Lama, Pasar Air Panas and Pasar Sentul while the receiving rivers of wastewater are

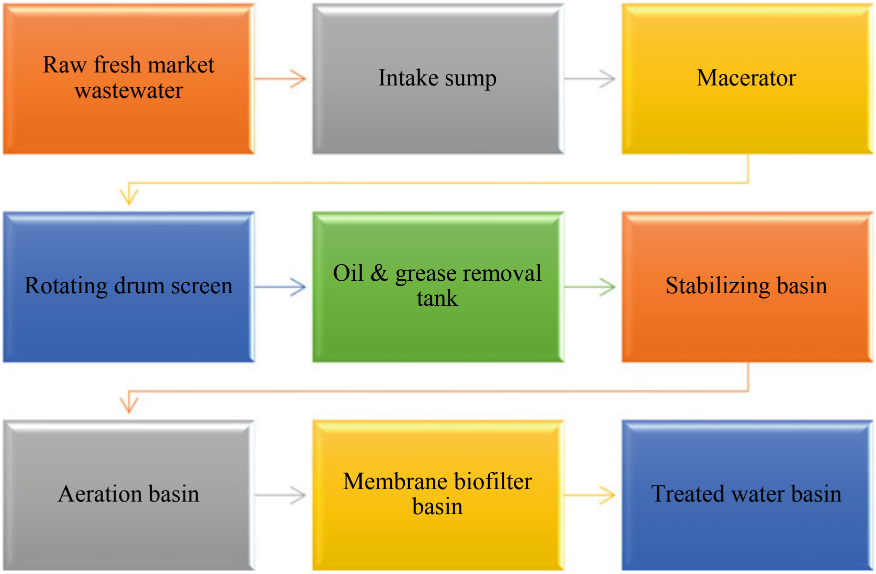

Fig. 4.1 Process of fresh market wastewater treatment plant in Kuala Lumpur

Jinjang River (Pasar Harian Selayang and Pasar Borong KL), Klang River, Bunus River and Gombak River, respectively (KLCH 2016).

The membrane biofilter was chosen for the fresh market wastewater. The processes of water treatment plants are demonstrated in Fig. 4.1.

Raw fresh market wastewater flows into the intake sump. In this stage, the wastewater flows to the coarse and fine screen to prevent rubbish and debris to enter the sump. There are three submersible pumps for duty and standby. The basin depth is 5.0 m which is controlled by ultrasonic level sensor. From inlet sump, the wastewater will flow into inline macerator to grind large solids into small particles with size 4–5 mm. Next, the fresh market wastewater flows to rotating drum screen to screen the small particles with the size of 0.5 mm before flowing into oil and grease removal tank. The purpose of this tank is to remove floating free oil with the disposal of oil and grease had been recycled for fuel oil by licensed contractor registered with Department of Environment Malaysia. The stabilizing basin is vital to store effluent with supplied oxygen. The three submersible pumps were used, and the basin depth was controlled by ultrasonic level sensor with maximum depth of 3.5 m with hydraulic retention time of three to four hours to homogenize the pollutant loading. The diffuser membrane material by Ethylene–Propylene–Diene Monomer (EPDM) was used with 1–3 mm bubble size, and air flow rate is 5–8 m^3/h. The fresh market wastewater from stabilizing basin will flow into aeration basin. This basin is important to remove suspended solid, COD and BOD with the presence of bubble diffuser to increase biological oxidation process. The oxidized effluent will decant into membrane biofilter basin by outlet opening. The basin depth is 3.5 m with hydraulic retention time of four to five hours and rate of aeration is 12.5 m^3/min.

The next process is wastewater flow into membrane bioreactor basin. The set packages of membrane bioreactor are used in this stage with hooked up to the permeate suction pipe plastic hose, and the water sucked is through the membrane in membrane bioreactor by permeate pump. Three self-priming pumps/trains were used for duty or standby in this stage. Next, the wastewater is re-supplied to the bottom membrane bioreactor through diffuser located underneath the membrane bioreactor package. The air bubble blows through membrane and loosen the dirt with drop down which accumulates as sludge. The membrane material is high-density polyethylene with hollow fibre type. The size of membrane bioreactor is 0.4 μm with allowable pH range of 2–13 and optimum temperature of 13–35 °C for operating condition. Finally, the wastewater flows to treated water basin. This basin is used to store the wastewater before discharge into outlet drain which is used in the backwash operation. This stage was controlled by float sensor with the basin depth of 3.5 m (KLCH 2016).

Figure 4.2 shows the installation process while Fig. 4.3 shows the maintenance process for membrane bioreactor. Sludge digesting basin is needed to store accumulated sludge in which the interval of sludge desludging depends on effluent quality. The basin depth is 3.5 m. The sludge disposal is collected and transferred to Jinjang Transfer Station. Washing basin is used during membrane bioreactor cleaning. The membrane bioreactor frame is soaked into this basin. Chemical tank contains sodium hypochlorite and oxalic acid which was supplied to the basin using chemical dosing metering pump. The depth of washing basin is 3.5 m. The monitoring work needs to be conducted to maintain good effluent quality by checking the control panel, data collection and water flow rate and gauge.

The summary of influent and effluent water quality was recorded with the data collected on 22 January 2016 as shown in Table 4.3.

Muntinlupa City Public Market in Muntinlupa City, Metro Manila, Philippines is the largest public market in the metropolitan area with 1448 stalls and 24 h operating per day. The sewage, grease and wastewater from washing the meat and fishes that discharge to a septic tank produce odour that is uncomfortable to the customers. The partially treated fresh market wastewater then flows to the Laguna Lake which

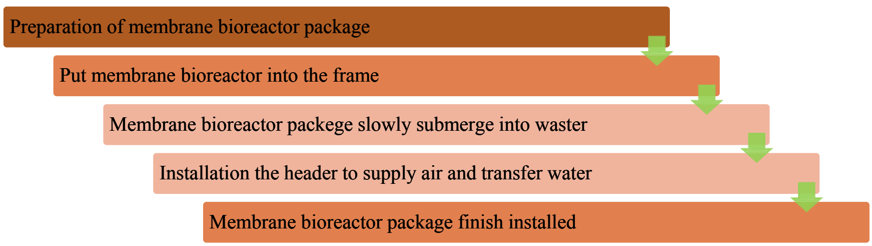

Fig. 4.2 Installation process of membrane bioreactor

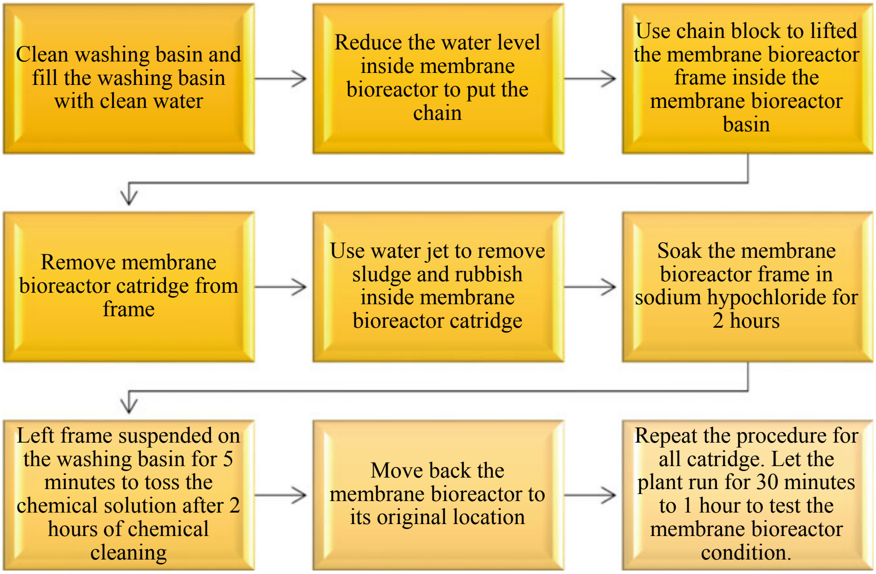

Fig. 4.3 Maintenance process of membrane bioreactor

Table 4.3 The parameter before and after treatment

Parameter	Inlet sump	Final treated wastewater	[a]Class IIB
Temperature (°C)	30.7	30.6	–
BOD_5 at 20 °C (mg/L)	37.2	2.9	3
COD (mg/L)	88	8.0	25
TSS (mg/L)	41	3	50
Turbidity (NTU)	42	0.3	50
Dissolved oxygen (DO) (mg/L)	1.3	7.5	5–7
E. coli (MPN/100 mL)	n.d.	n.d.	–

[a]n.d. is not detected; Interim National Water Quality for Malaysia—Class IIB: Recreational use with body contact

is main inland sources of drinking water and freshwater fish at Manila. A sewage treatment system for this fresh market wastewater was constructed and implemented by the government and Philippine Sanitation Alliance (PSA)'s precursor, to reduce the amount of pollution flow into the Laguna Lake, to clean up surrounding area and to build the small scale of wastewater treatment system that could be replicated in other markets (PEN 2008; CAP 2011).

The system in Muntinlupa City Market consists of several process flows as shown in Fig. 4.4. The system was located under the parking lot due to limited ground space and was designed to treat up to 210 m^3 of fresh market wastewater per day. The bar screen is located at each main inlet area to filter large floatable debris such as garbage, fish or meat trimming before flowing to the lift station which is a simple chamber containing aired automatic pumps to collect wastewater to the equalization tanks. This equalization tank is the point that wastewater retention and allows relatively constant flow of wastewater to proceed to the next chamber. After that, the wastewater flows to the anaerobic baffled reactor which is the main treatment technology used for this project. This multi chambered (this project used four chambers anaerobic baffled reactor) is closed without natural air and the wastewater flows slowly up and back down in the pipes, through the identical chambers, each time entering the chamber at bottom, where it passes through the accumulated sludge. The settleable solids in the sludge and anaerobic bacteria in the sludge degraded the harmful organic and chemical components in wastewater (PEN 2008; CAP 2011).

The up-flow anaerobic sludge blanket is a single tank with baffled near the top of wastewater level in which the wastewater flows upward through the blanket, and the sludge was settling using gravity with the aid of flocculant. The sludge then decomposes by anaerobic bacteria. The degradation is methanogenic (methane produced) and produces carbon dioxide. The bubbles rise upward and provide natural mixing to the wastewater above the sludge layer. The baffles guide the gas bubbles towards

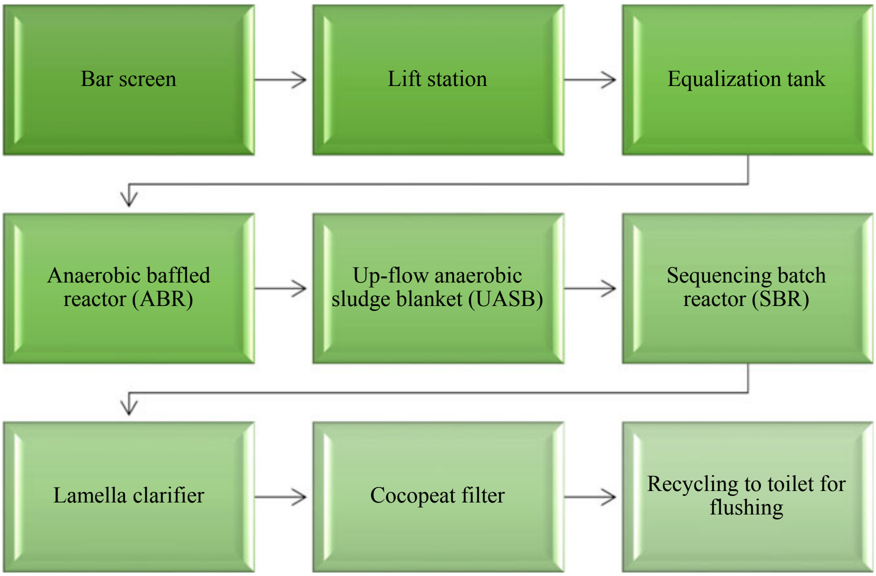

Fig. 4.4 Process of fresh market wastewater in Muntinlupa city market

the top and centre of the tank which is known as ‘gas cap’. Indirectly, the gas caps prevent remaining particles to enter the next tank. This tank can reduce 50–70 % BOD, COD and TSS (PEN 2008; CAP 2011).

The sequencing batch reactor is the chamber that treats fresh market wastewater in batches and not continuously with activated sludge process which consists of open-air tank. The aeration from air injection pipes at the bottom tank with specific rate increases aerobic bacteria growth rate. These bacteria consume organic compound and nutrients present in wastewater, were clumped together and suspended in the tank. The process reduced the amount of BOD and COD (PEN 2008; CAP 2011).

The stages for sequencing batch reactor are

i. Filling—filled with pre-treated from up-flow anaerobic sludge blanket,
ii. Reaction—bacteria react with the aerated wastewater, then flocs,
iii. Settling—when aeration stopped, the flocs are allowed to settle and give clear effluent,
iv. Decanting—effluent pumped out of the tank from the top of wastewater (it does not disturb the sludge blanket at the bottom),
v. Idling—the sequencing batch reactor idles until for the next cycle and,
vi. Sludge wasting—the excess sludge is periodically removed from the tank.

The cocopeat filter was used for this site for about one year as demonstration. The treated wastewater is dripped through the cocopeat, to ensure the wastewater is clean for reusing or for discharging the effluent. The effluent was reused for toilet flushing for about a year and now currently used for cleaning the streets.

San Fernando Public Market in San Fernando City at La Union, Philippines runs a small-scale wastewater treatment system. The main city market with 700 stalls (sometimes up to 900 stalls on the market days of Wednesday, Saturday and Sunday). The wastewater from septic tank then flows to the drainage canal which pose a significant threat to the coastline city. Thus, the wastewater polluted the nearby beaches and threatened human health in the surrounding area. The newly constructed sewage treatment system was used to clean up the coastline at San Fernando City with technical assistance from the United States Agency for International Development (USAID) funded Environmental Cooperation-Asia project (PEN 2008; CAP 2011).

The flow process of wastewater treatment system at San Fernando Public Market is illustrated in Fig. 4.5. The chlorination tank consists of a tank and supplied chlorine to kill the remaining pathogens or bacteria in the wastewater. After dissipation, the effluent can be recycled for toilet flushing or discharged.

Sta. Ana Public Market in Manila City, Metro Manila, Philippines which is alongside the Pasig River has 220 stalls. This public market discharges the partially treated wastewater to the river, thereby increasing the pollution in Pasig River which is known as the most polluted river in the world. The river is considered as ‘biologically dead’ due to the combination of this public market wastewater and domestic wastewater. Thus, PSA, Rotary and government worked together to clean the river by constructing a sewage treatment system for this public market wastewater. This wastewater treatment system also can be used as a model that can be replicated in other public markets along the river (PEN 2008; CAP 2011).

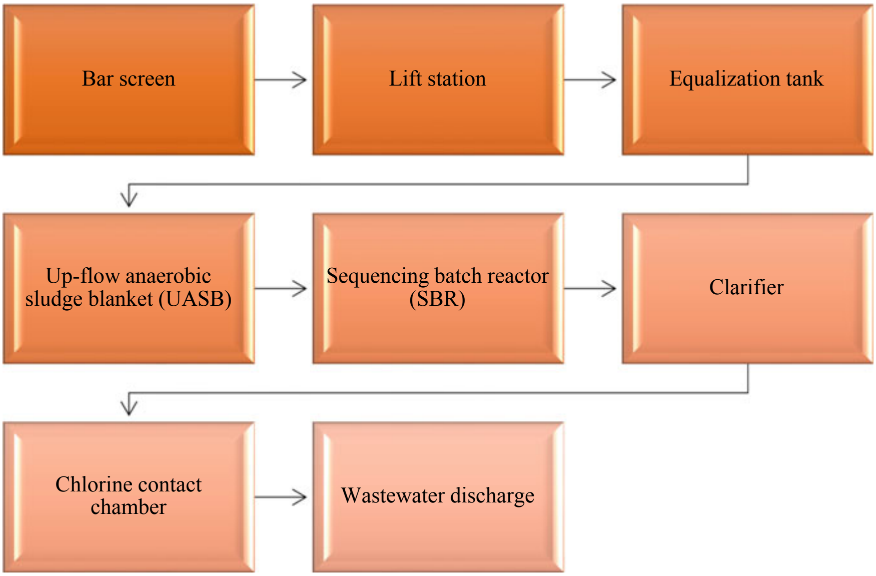

Fig. 4.5 Process of fresh market wastewater in San Fernando public market

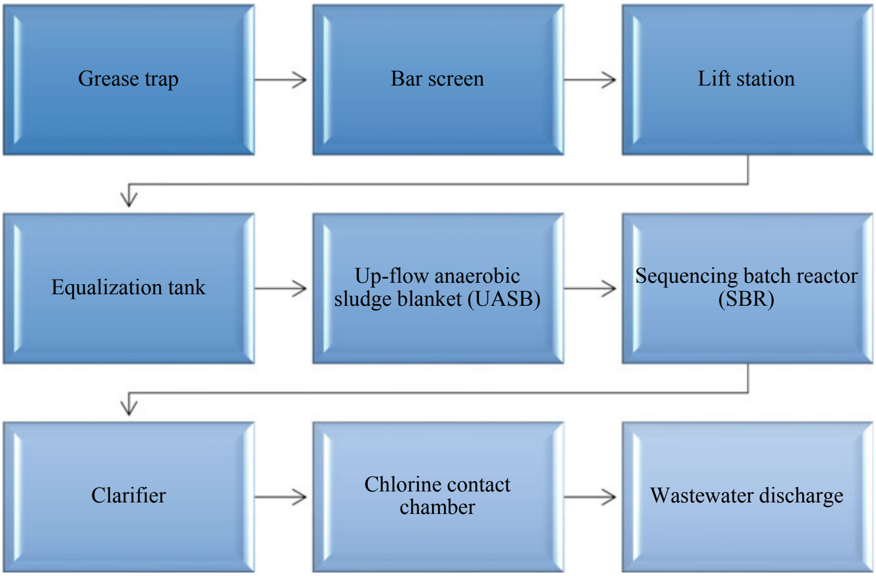

Fig. 4.6 Process of fresh market wastewater in Sta. Ana public market

Figure 4.6 shows the treatment flow for Sta. Ana Public Market. The grease trap is a simple tank consisting of one or two chambers, with a position deep below the anticipated wastewater level. The floating grease was trapped while the wastewater will exit through the pipes (Rahmat et al. 2018; Al-Gheethi 2019).

4.6 Treatment Technologies in Case Study in Laboratory Experiment

There are several case studies of fresh market wastewater treatment technology using laboratory batch experiment which are

(a) Effective microorganisms for preliminary treatment.
(b) Phycoremediation process by microalgae *Scenedesmus sp*.
(c) Sequencing batch reactor.
(d) Modified sand filtration for preliminary treatment.

Shahbuddin et al. (2018) use effective microorganisms to treat fresh market wastewater during preliminary treatment. Effective microorganisms are nature-based solution for sustainable water management and popular media due to its low operational cost and synergistic effects to the environment. The effective microorganisms are widely applied in many countries as a method for wastewater treatment. The effective microorganisms are antioxidant, able to inhibit harmful bacteria substance and enhance the proliferation of beneficial microorganisms and fungi to restore and balancing natural system.

The wastewater sample from Selayang Public Market located at Bandar Baru Selayang, Selangor were collected for the study. This fresh market wastewater was discharged directly to the river with intersection of the most critical sources of untreated wastewater from two rivers, Batu River and Jinjang River. The wastewater samples were collected for one hour interval starting from 7 am to 12 pm by using 2 L clean plastic sampling bottles and preserved by adding sulphuric acid (H_2SO_4) to reduce the pH to 2 or less. The parameters from fresh market wastewater that were tested are pH, $DOBOD_5$, CODTSS and ammoniacal nitrogen ($NH_3{-}N$). The wastewater samples were mixed by adding all of the samples in each interval, in a beaker with magnetic stirrer. 100 mL of effective microorganisms were then added into 1 L of wastewater sample.

As the result, flow rate at 8 am show highest amount with 0.015 m^3/s and lowest at 10 am, and it can be assumed the peak hour of this market activities is at 8 am The highest BOD_5, COD, TSS and $NH_3{-}N$ is 423 mg/L, 1566 mg/L 933.3 mg/L and 0.14 mg/L, respectively, with the range of pH and DO are 6.79–7.18 and 7.38–7.53 mg/L. Shahbuddin et al. (2018) revealed the bacteria existed in the wastewater is not tolerant in lower dissolved oxygen level which they need to survive and decompose the organic compound. The nitrogen is vital for plants and animals in the formation of protein for cell growth.

Table 4.4 The differences before and after preliminary treatment by effective microorganisms

Parameter	Before	After
pH	8.96	8.26
DO (mg/L)	7.74	7.71
BOD_5 (mg/L)	549	182
COD (mg/L)	1328	1074
TSS (mg/L)	466.67	200.00
$NH_3{-}N$ (mg/L)	0.07	0.01

Table 4.4 shows the data collected during preliminary treatment by effective microorganisms. There are no significant changes in DO using effective microorganisms. However, the effective microorganisms are successful to reduce BOD_5 with 66.8 %, COD with 19.1 %, TSS with 57.1 and 85.71 % reduction for $NH_3{-}N$. Hence, this preliminary treatment is good to protect membrane for filtration in treatment plant.

Jais et al. (2015) used phycoremediation by microalgae to treat fresh market from Parit Raja Public Market, Batu Pahat. Microalgae are widely used as a medium to treat wastewater such as municipal wastewater, industrial wastewater and animal wastewater. However, the lacking relevant information about wet market wastewater treatment using microalgae led to this study. 10 L of wastewater was collected around 9 am. The characteristics of wastewater such as turbidity, BOD, COD, TSS, TN, Total Phosphorus (TP), O&G, Zn and Fe were collected. Microalgae *Scenedesmus* sp. were chosen as a phycoremediate agent to treat wastewater. The culturing of microalgae was done using Bolts Basal Medium (BBM). To get the optimum concentration of microalgae to phycoremediate wastewater, five sets of wastewater samples were prepared with adding different microalgae *Scenedesmus sp*. concentration (6.50×10^5, 49.88×10^4, 34.75×10^4, 19.63×10^4 and 4.50×10^4 cell/ml). Nutrients and elements amount were measured on zeroth, second, fourth, sixth and eighth day for each sample. The efficiency removal in percentage was used to determine the efficiency of phycoremediation process.

The fresh market wastewater characteristics at Parit Raja Public Market are turbidity (66.0 ± 8.9 NTU), BOD (89 ± 3.61 mg/L), COD (456 ± 8.19 mg/L), TSS (132.3 ± 1.7 mg/L), TN (36.9 ± 0.5 mg/L), TP (1.61 ± 0.13 mg/L), oil and grease (5.22 ± 0.07 mg/L), Zn (0.312 ± 0.0021 mg/L) and Fe (1.071 ± 0.0010 mg/L). Microalgae are known as sequester elements due to their ability to remove elements in wastewater by absorbing the elements for their growth. Nitrogen and phosphorus are primary concern to remove by phycoremediation in these wastewater samples. Highest efficiency removal of nutrients and elements by different initial microalgae concentrations are as in Table 4.5. 6.50×10^5 cell/mL of microalgae absorbed maximum amount of TN by eliminating 74.77 % from wastewater while the TP was reduced up to 82.17 % removal by initial concentration, 34.75×10^4 cell/mL of microalgae. 49.88×10^4 cell/mL of microalgae can remove 65.76 % of Fe while 6.50×10^5 cell/mL removed 84.14 % of Zn. As conclusion, the microalgae *Scenedesmus* sp. have good ability to remove nutrients and elements within 8 days experiment, and concentration 49.88×104 cell/ml of microalgae *Scenedesmus sp*. was chosen

Table 4.5 Highest efficiency removal of nutrients and elements by phycoremediation

Nutrients	Highest efficiency removal (%)				
	6.50×10^5 cell/mL	49.88×10^4 cell/mL	34.75×10^4 cell/mL	19.63×10^4 cell/mL	4.50×10^4 cell/mL
TN	74.77	65.32	46.03	51.52	50.71
TP	79.75	76.77	82.17	82.07	79.29
Elements	*Highest efficiency removal (%)*				
	6.50×10^5 cell/mL	49.88×10^4 cell/mL	34.75×10^4 cell/mL	19.63×10^4 cell/mL	4.50×10^4 cell/mL
Fe	55.77	65.76	59.79	61.21	45.60
Zn	84.14	82.12	79.67	81.33	71.94

as the most efficient concentration due to the highest efficiency removal of nutrients and elements (Jais et al. 2015).

Danial et al. (2016) used a sequencing batch reactor to remove nutrients from greywater at Peladang Public Market, Skudai. Bio. The sludge was obtained from wastewater treatment plant (Taman Harmoni Wastewater Treatment Plant) as inoculum. Sequencing batch reactor was built by fabricated transparent glass cylinder with a total volume of 4 L of wastewater. The system was operated under alternating anoxic-aerobic condition within 28 days with activated sludge process (MLSS) maintained at 3000–5000 mg/L. The sequencing batch reactor was equipped with two peristaltic pumps with (35 L/min of air feeding) mechanical agitator (30 rpm) to mix well with three cycles per day (1.2 days of hydraulic retention time). The pH was set at a range of 7.0–7.5 with natural room temperature.

The system of this sequencing batch reactor consisted of four steps:

(1) Filling—the filling process is done within half an hour, and the air pump was stopped to promote anoxic condition and continued for two hours after feeding.
(2) React—the aeration using mechanical agitator and air pump automatically occurred for four hours and offed for one hour.
(3) Settling—sedimentation or clarification.
(4) Draw—decanting occurs when 1000 mL of supernatant was removed from the sequencing batch reactor within half an hour.

The characteristics of wastewater are COD was 1708 mg/L, TSS was 140 mg/L, TN was 66 mg/L, TP was 288 mg/L, while NH_4-N was 98 mg/L. The maximum effluent of COD is 97 mg/L which reduces up to 94 % using the sequencing batch reactor. The TSS also removed 71 %. The efficiency removal of TN, TP and NH_4-N are 88 %, 67 % and 93 %, respectively. It is proven that sequencing batch reactor effectively removes nutrients and promotes biodegradation of organic matter for fresh market greywater.

The study of modified sand filtration as a preliminary treatment for fresh market wastewater was conducted by Saad et al. (2016). Sand filtration is one of the oldest wastewater treatment technology which is of low cost and maintenance and high treatment efficiency. The four sand filtration systems were constructed in transparent plastic column with the size 260 mm of height, 80 mm of top diameter and 24 mm for bottom diameter. A hole at the bottom is presented as a channel for effluent disposal. There are three media used in this study which are fine sand, coarse sand and activated carbon (rice husk and coconut shell) with 0.06 mm, 2.00 mm, and 0.08 mm in their effective size, respectively. Four types of sand filtration were used in this study such as (a) sand filtration, (b) sand filtration with coconut shell, (c) sand filtration with rice husk and (d) sand filtration with rice husk and coconut shell (Fig. 4.7).

The characteristics of wastewater are BOD 137.03 mg/L, COD 350.27 mg/L, TSS 126.4 mg/L and NH_3-N 45.81 mg/L while efficiency removal of four types

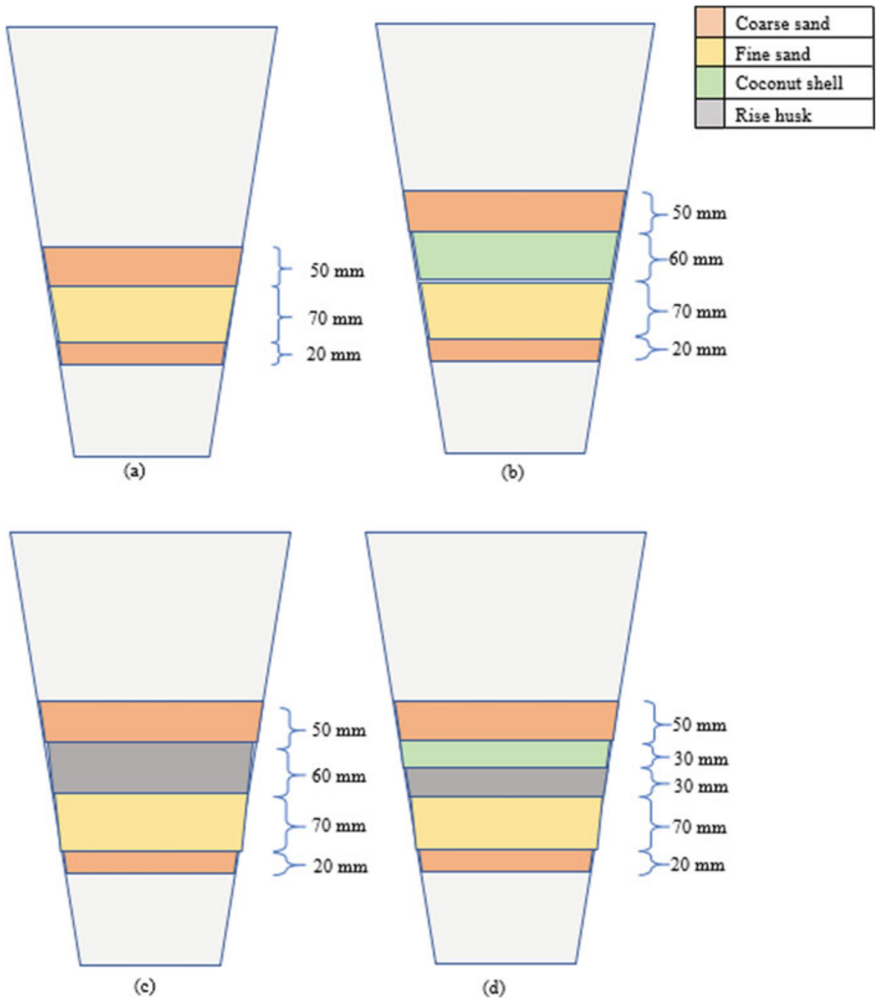

Fig. 4.7 Schematic diagram of sand filtration system

Table 4.6 Efficiency removal of organic compound and nutrient using sand filtration treatment system

Parameter	Efficiency removal (%)			
	Sand filtration	Sand filtration with coconut shell	Sand filtration with rice husk	Sand filtration with coconut shell and rice husk
BOD	41.8	54.1	86.2	69.2
COD	46.7	53.6	84.4	66.8
TSS	0.0	40.5	63.0	32.3
NH_3-N	43.2	76.1	87.6	81.5

of treatment system is recorded in Table 4.6. The sand filtration with the presence of rice husk indicates 86.2 and 84.4 % of BOD and COD removal,respectively. It shows that organic matter from fresh market wastewater was removed. The TSS and NH_3-N in wastewater also indicated removal as 63.0 and 87.6 %, respectively. Overall result is successful in efficiency reduction up to 30 % except TSS using sand filtration. This study revealed that sand filtration with addition of activated carbon (rice husk) shows potential in eliminating the amount of organic compound and nutrients. Therefore, the addition of activated carbon in filtration method can be one alternative preliminary treatment system for fresh market wastewater.

4.7 Impact and Challenges

The several projects of wastewater treatment system at fresh markets area are still operating and maintained. The treatment system is still functioning properly and serves as a significant model. The Muntinlupa market, for example showed an innovative project since the system is underground due to the limited space. The volume of wastewater treated is also a reasonable sum. The cost of operating and maintaining is also lower compared to the conventional systems. The treated wastewater usage as toilet flushing and street cleaning also saves money on water bills. The combination of aerobic and anaerobic treatment process allows the amount of BOD, COD and TSS meet to allowable standard after treatment. However, the treatment system still faces challenges. For example, in Muntinlupa Public Market, several pumps broke down and need to be replaced which takes time. In San Fernando Public Market, the treatment system is always and easily clogged with the trash and grease due to poor design and construction (PEN 2008). Overall, the several wastewater treatment systems that had done successfully improved the wastewater quality by eliminating the largest and highest parameter strength of wastewater into important water bodies such as Jinjang River, Klang River, Bunus River, Gombak River, Pasig River, Laguna Lake and coastlines in Philippines.

Acknowledgements The authors gratefully acknowledge the Ministry of Higher Education of Malaysia for the financial support under the Prototype Research Grant Scheme (PRGS) vot No. G004 and My PhD.

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Chapter 5
Microalgae Production in Fresh Market Wastewater and Its Utilization as a Protein Substitute in Formulated Fish Feed for *Oreochromis* Spp.

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Abstract Rapid growing of human population has led to increasing demand of aquaculture production. *Oreochromis niloticus* or known as tilapia is one of the most globally cultured freshwater fish due to its great adaptation towards extreme environment. Besides, farming of tilapia not only involves small scales farming for local consumption but also larger scales for international market which contributes to a foreign currency earning. Extensive use of fishmeal as feed for fish and for other animals indirectly caused an increasing depletion of the natural resource and may consequently cause economic and environmental unstable. Microalgae biomass seems to be a promising feedstock in aquaculture industry. It can be used for many purposes such as live food for fish larvae and dried microalgae to substitute protein material in fish feed. The microalgae replacement in fish feed formulation as protein alternative seem potentially beneficial for long term aqua-business sustainability. The present chapter discussed the potential of microalgae as an alternative nutrition in fish feed formulations, specifically Tilapia.

Keywords Aquaculture · Fish feed · Microalgae · Freshwater fish · Tilapia

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A. A. S. Al-Gheethi et al. (eds.), *Prospects of Fresh Market Wastes Management in Developing Countries*, Water Science and Technology Library 92,
https://doi.org/10.1007/978-3-030-42641-5_5

5.1 Introduction

The farming of animals and plants in aquatic environment or known as aquaculture is becoming an important industry in the world to remunerate the declining of capture fisheries and to reduce the pressure on marine resources (Yusoff 2003). As reported by Food and Agricultural Organization (2001), the world aquaculture production is growing more than 10% every year. In contrast, the growth of capture fisheries and livestock increase only by 1.5% and 3%, respectively. The rapid growing of this industry involving small scales aquaculture for local consumption and large scales for international markets indirectly contribute to foreign currency earning. According to the Canadian Institute for Climate Studies (2000), aquaculture practices not only contribute to the increasing of national food production but also act as an alternative in overcoming the reduction of fish stock due to overexploitation of fishing activities.

In Malaysia, aquaculture industry is being promoted as one of the most important engines of economic growth for the country (Yusoff 2015). Aquaculture practices was first developed in 1920s in Peninsular Malaysia but only begun to grow in early 1930s in East Malaysia. It began with the freshwater aquaculture and brackish water aquaculture in the late 1930s. Malaysia is surrounded by good climate conditions which allow for year-round aquaculture production. In spite of the raining season from November to January in the east coast of the peninsular, Malaysia is located in a low-risk area of natural disasters. This advantage makes Malaysia a highly potential country in the aquaculture industry. Williams (2003) reported that the consumption per capita fish nationally has reached up to 57.7 kg, which is higher than the Asian average of 28.0 kg and also the world's average of 15.8 kg. In 2014, Malaysia was among the highest in fish consumption in Asia, annually at 56.5 kg per capita (Othman et al. 2017). Mazlan (2000) stated that approximately 60–70% of the total protein intake is in the form of fish and fisheries products. This shows that the main role of aquatic resources is in the nutritional intake of Malaysian population. An average of 20% of Malaysian's dietary food is seafood and this trend remains unchanged despite the availability of chicken (Othman et al. 2017). The same study also stated that aquaculture is the main source of protein for 30.75 million inhabitants in the country. It may be due to the declination in production from capture fisheries (Othman et al. 2017). In 2016, contribution of aquaculture was 506,454 tonnes, valued at MYR 3.3 billion (Department of Fisheries 2016). Apart from that, aquaculture also provides employment, business and investment opportunities in the country. In 2015, there were 23,832 aqua farmers in Malaysia, mainly in freshwater sector and the majority are owner-operators (Department of Fisheries 2016). Malaysia aquaculture practices is being carried out in earthen ponds, floating sea, river cages and tanks. Currently, there are three main practices which are in freshwater, brackish water and marine aquaculture (Hamdan et al. 2015), with the most preferred farmed fish are *Clarias* catfish and tilapia (*Oreochromis niloticus*) (Othman et al. 2017). The present chapter discussed the potential of microalgae as an alternative nutrition in fish feed formulations, specifically Tilapia.

5.2 *Oreochromis* spp.

Oreochromis spp. or locally known as tilapia are freshwater fish classified under family Cichlidae. According to Trewaves (1983), this freshwater fish is exclusively related to Africa and Middle East and have been called the "Saint Peter's fish" referring to biblical passages about the fish being fed to the multitudes (Popma and Masser 1999). Illustration from Egyptian tombs suggested that tilapias has been cultured more than 4000 years ago and 1000 years before carp fish was introduced in China (Balarin and Hatton 1979). Moreover, tilapia was introduced into many sub-tropical, tropical and temperate regions of the world during the second half of twentieth century (Pillay 1990). Tilapia have fairly conventional and laterally compressed deep body shapes (Ross 2000). The body of tilapia is covered with relatively large and cycloid scales which are not easily dislodged. The dorsal and anal fins of this fish consist of hard spines and soft rays (Ross 2000). Tilapia bodies are generally characterized by vertical bars, with relatively subdued colours and with little contrast over the body colours. Tilapia also have well-developed sense organs represented by prominent nares and a clearly visible lateral line. The fish possess an excellent visual capability (El-Sayed 2016).

Tilapia offers a great possibility commercialization because of their extreme culture adaptability (Amal and Zamri-Saad 2011) which are fast-growing with firm, white flesh and able to survive in poor water conditions, eat wide range of food types, breed easily with no need for special hatchery technology (Nandlal and Pickering 2004) and also feed at the base of the aquatic food web (Beveridge and Baird 1998). In addition, tilapia species can tolerate a wide range of environmental conditions (Nandlal and Pickering 2004). According to FAO (2004), tilapias are among the most cultured fish worldwide. The production of tilapias made it one of the most important species for the twenty-first-century aquaculture (Fitzsimmons 2000), which commercially increase in more than 100 countries (Shelton and Popma 2006). The estimated global tilapia production for the year 2000 was 1.5 million metric tonnes (MT) compared to 28,260 metric tonnes in 1970. Furthermore, China alone produced 706,585 MT of cultured tilapia in 2000 which represent 50% of the total world production (FAO 2004; El-Sayed 2006). On the other hand, the global production of cultured tilapia in at least 85 countries exceeded 2.5 MT in 2007 (FAO 2009). Most of the tilapia production from Asia countries contributes about 80% of the global production. Nine top countries which contribute to high production of tilapia from 1980 to 2007 are China (including Taiwan), Egypt, Philippines, Indonesia, Thailand, Brazil, Honduras, Colombia and Malaysia (Table 5.1). Three species of tilapia are used in aquaculture worldwide, namely, *Oreochromis niloticus* (Nile tilapia), *Oreochromis mossambicus* (Blue tilapia) and *Oreochromis aureus* (Mozambique tilapia) (FAO 2001).

In Malaysia, tilapia farming was introduced to fish farmers in 1952 and the main species cultured was *Oreochromis mossambicus* (Department of Fisheries 2015). However, in 1980, this practice was converted to commercial culture and *Oreochromis niloticus* became the main species used until present (Department of

Table 5.1 Tilapia production of 9 top countries from 1980 to 2007 (FAO 2009)

Country	Production in tonnes (t)						
	1980	1985	1990	1995	2000	2005	2007
China	44,832	76,542	159,313	361,346	598,109	928,163	1,210,167
Egypt	9,000	22,346	24,916	21,969	157,425	217,019	265,862
Philippines	13,214	42,640	76,142	81,954	92,575	163,004	241,183
Indonesia	14,901	29,302	53,768	74,125	85,179	189,570	248,305
Thailand	8419	16,542	22,895	76,383	82,581	203,911	190,258
Brazil	Nr	Nr	Nr	12,014	32,459	67,851	95,091
Honduras	6	35	120	172	927	28,376	28,356
Colombia	93	300	2040	16,057	22,870	27,953	27,960
Malaysia	366	314	1145	8866	18,471	28,635	32,258

Nr: Not recorded

Fisheries 2015). Due to large scale availability of diverse freshwater bodies such as lakes, reservoirs, ex-mining pools and irrigation canals, the potential for tilapia farming in Malaysia is high (Hamzah et al. 2014; Shrestha and Pant 2012). Hamzah et al. (2014) reported that *Oreochromis niloticus* is widely cultured in ponds, cages, tanks and pen culture system. In 2015, the recorded production in total for the year 2013 was 132,892 MT with value of RM 1,225 million and approximately 44,099 MT of the total amount was Tilapia with the value worth RM 329 million (Department of Fisheries 2015). Pradeep et al. (2010) stated that Nile tilapia contributes to the 80% of total tilapia production and 20% of it is from Black tilapia.

5.3 Characteristics of Microalgae

The increasing needs for protein and the high cost of fishmeal have led to the requirement of finding new alternative to replace fishmeal (Sirakov et al. 2015). The most accessible and inexpensive food component that can replace fishmeal is microalgae. Microalgae is recognized as one of the oldest life forms on earth and it is a promising source of biomass (Taelman et al. 2013). Microalgae also produce more biomass than the terrestrial plants per unit area due to its higher efficiency in photosynthesis (Chisti 2008; Packer 2009; Mata et al. 2010).

Production of microalgae does not compete directly with food crops because they can be easily cultivated in almost any ecosystems including freshwater, brackish water or saltwater depending on the species. Microalgae uses carbon dioxide during photosynthesis process with the aid of other excess nutrients such as phosphorus and nitrogen (Mohamed et al. 2018; Apandi et al. 2019a). According to Clarens et al. (2010), these characteristics provide microalgae with capability to sequester carbon dioxide and purify nutrient-rich wastewater (Yaakob et al. 2019; Apandi et al. 2019b).

Microalgae is also known as an essential food source for all stages of marine bivalve mollusc, larval stages of marine gastropods and larvae of several fish species due to the large amounts of useful carbohydrates, proteins and antioxidants (Muller-feuga 2000; Al-Gheethi et al. 2019). Moreover, microalgae contain a great essential of polyunsaturated fatty acids such as omega-3 and omega-6. Good pigments, minerals and vitamins content in microalgae are also the beneficial characteristics that could make this microscopic plant the best alternative for protein replacement in fish feed (Maizatul et al. 2017).

5.4 Physicochemical Properties of Microalgae

Microalgae could store high concentration of carbohydrates because it shows a relatively high photo conversion efficiency which are more than 50% of dry weight (Ho et al. 2012; Jais et al. 2017), relevant biological functions in microalgae cells as a storage, protection and structural molecules (Arad and Levy-Ontman 2010). The composition of carbohydrates depends on the species. For examples, cyanobacteria synthesize glycogen ($\alpha - 1, 4$ linked glucan), red algae floridean synthesize starch (hybrid of starch and glycogen) and green algae synthesize amylopectin-like polysaccharides (starch) (Markou and Georgakakis 2011). Several species of microalgae such as *Porphyridium cruentum* (40–57%) and *Spirogyra* sp. (33–64%) have a naturally higher content of carbohydrate (Harun et al. 2010). The carbohydrate content of microalgae can be modified by cultivation and environmental factors, salt stress, light intensity and temperature. Moreover, the type of carbon source and metabolism process is also one of the major factors that influence sugar content (Harun et al. 2010; Pahazri et al. 2016).

In 1950s some species of microalgae were introduced as a source of protein (Soletto et al. 2005). Protein content in microalgae is in the form of amino acids which cannot be synthesized in human or animal bodies. A study by Becker (2007) reported that microalgae are good quality of non-conventional protein sources thus suitable to be used as protein alternatives in fishmeal.

Three species that are commonly used for protein production are *Chlorella* (55% protein content), *Spirulina* (*Arthrospira*) (65% of protein) and *Dunaliella* (57% of protein content) (Gonzalez-Benito et al. 2009). *Spirulina* has received more attention due to its good quality and quantity of protein reaches 70% of dry weight. According to Andrade et al. (2018). *Spirulina* proteins that are rich in essential amino acids has been used for a long time as protein supplement and also to manufacture healthy foods. This finding is supported by Becker (2007), who stated that amino acids (i.e., lysine, methionine, tryptophan, threonine, valine, histidine and isoleucine) in microalgae are comparable with the conventional protein sources such as egg and soybean (Table 5.2).

In addition to high and good quality of carbohydrates and protein content, microalgae have also been reported to contain high antioxidants. Astaxanthin, β-Carotene

Table 5.2 Amino acid profile of different microalgae as compared with conventional protein sources (g per 100 protein) (Becker 2007)

Source	Amino acid content (g per 100 g protein)						
	Ile	Val	Lys	Met	Trp	Thr	His
Egg	6.6	7.2	5.3	3.2	1.7	5.0	2.4
Soybean	5.3	5.3	6.4	1.3	1.4	4.0	2.6
Chlorella vulgaris	3.8	5.5	8.4	2.2	2.1	4.8	2.0
Dunaliella bardawil	4.2	5.8	7.0	2.3	0.7	5.4	1.8
Scenedesmus obliquus	3.6	6.0	5.6	1.5	0.3	5.1	2.1
Arthrospira maxima	6.0	6.5	4.6	1.4	1.4	4.6	1.8
Spirulina platensis	6.7	7.1	4.8	2.5	0.3	6.2	2.2
Aphanizomenon sp.	2.9	3.2	3.5	0.7	0.7	3.3	0.9

and other carotenoids are examples of antioxidants derived from microalgae. Antioxidant is a good substance to protect cells from free radicals (Nimse and Pal 2015). The researchers also discovered that antioxidants of microalgae have stronger effect than vitamin E but weaker than synthetic antioxidants such as butylated hydroxytoluene or butylated hydroxyanisole. However, due to health concern and perceptions of synthetic products, demand for natural products is increasing. Hence, most manufacturers and producers begin to replace or incorporate synthetic materials to natural ingredients especially in the manufacturing of food and nutraceutical products (Munir et al. 2013). In addition, the natural antioxidant substances have higher bioavailability and a better protective effect than synthetic antioxidant substances (Spolaore et al. 2006).

Polyunsaturated Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the examples of omega-3 series long-chain polyunsaturated fatty acids. These microalgae-derived fatty acids are important products to be used in food and also animal feed industry and cannot be synthesized by human and animals (Pulz and Gross 2004). Therefore, it is recommended to be included in the daily diet (Simopoulos 2002). Spolaore et al. (2006) stated that some species of microalgae can produce fatty acids which vary not only between different species but also between different phases of development. These acids are beneficial for health of animals and humans because they contribute to the production of prostaglandins and thromboxane. Both substances are important for the reduction of cholesterol and triglycerides in blood; hence, prevent the cardiovascular diseases, atherosclerosis, skin diseases and arthritis (Gouveia et al. 2008).

Other important characteristic of microalgae is their colour. Some natural pigments of microalgae such as chlorophyll, phycobiliproteins, astaxanthin and carotenoids facilitate in absorption of sunlight and at the same time protect microalgae from sunrays (Gouveia et al. 2008). Moreover, these natural pigments also act as an antioxidant for the animal instead of consuming microalgae (Gouveia et al. 2008). Ferruzzi and Blakeslee (2007) have stated that chlorophyll is the primary photosynthetic pigment in all microalgae which contained 0.5–1.5% of dry matter.

These natural photosynthetic pigments are usually used in food products and pharmaceuticals industry due to its anti-inflammatory, wound healing and calcium oxalate controlling properties (Ferruzzi and Blakeslee 2007).

Phycobiliproteins pigment can usually be found in cyanobacteria and are deep coloured, water soluble and fluorescent. The examples are phycocyanin in Spirulina and *Aphanizomeni flos-aquae* (blue-green algae) (Gouveia et al. 2008). According to Pandey et al. (2014), phycobiliproteins have several characteristics such as antioxidants properties, anti- inflammatory and hepatoprotective observed from in vivo and in vitro study. These pigments are also used in clinical diagnostics as sensitive fluorescent indicators, as a natural colourant in cosmetics and nutrition, and in food products such as chewing gums, candies, dairy products, ice creams and refreshments (Gouveia et al. 2008).

Astaxanthin is a natural pigment that contains a powerful antioxidant that is more effective compared to vitamin C, E and other carotenoids such as β-carotene, lycopene, lutein and zeaxanthin. According to Mata et al. (2010), the astaxanthin content in *Haematococcus pluvialis* is between 1.5 and 3.0% of dry matter. The benefits of these pigments are as anti-inflammatory and immune-enhancing properties in both humans and animals. The same study also indicates that this pigment is commonly used in fish feed specifically for salmon feeds (Mata et al. 2010).

Carotenoids which are synthesized de novo primarily by photosynthetic organisms are naturally occurring pigments that have the ability to act as pro-vitamin A and contained in microalgae at the level 0.1–0.2% of dry matter (Becker 1993). Previous study by Gouveia et al. (2008) indicated that a diet rich in carotenoids may reduce the risk of having diseases involving free radicals such as atherosclerosis and cancer. According to Spolaore et al. (2006), β-carotene from *Dunaliella salina* has content up to 14% of dry matter. β-Carotene can be used in foods such as cheese and butter or margarine.

Microalgae are also an important source of vitamins such as tocopherols, ascorbic acid, B_1 (thiamine), B_2 (riboflavin), B_6 (phyridoxine), B_{12} (cobalamin), nicotinic acid, and biotin; and also macro minerals and micro minerals (Spolaore et al. 2006). Becker (1993) reported that 1 g of *Spirulina* contains 0.5–2.0 mg of vitamin B_{12} (cobalamin). Additionally, microalgae with high levels of vitamin B_{12} (cobalamin) and iron-like *Spirulina* makes it suitable as a nutrient supplement for vegan. According to Becker (2004), the vitamins content of microalgae is comparable to meat, bakery yeast, soybeans and also cereals. The vitamin content is commonly correlated with genotype, growth phase, nutritional status of microalgae and the light intensity during photosynthesis (Gouveia et al. 2008).

5.5 Microalgae as an Alternative Protein Source in Fish Feed

Global production of aqua feeds in 2003 was estimated approximately 19.5 MT and tilapia feeds contributes for approximately 8.1% of global aqua feed production in the same year (Tacon et al. 2006). The most common commercial tilapia feeds are dry sinking pellets and extruded floating pellets (Groenewald 2018). Food and Agricultural Organization (FAO 2019) reported that under semi-intensive farming systems, most tilapia farmers used formulated feeds while in intensive pond and tank culture systems or in cages, tilapia farmers mostly depend on commercial pelleted feeds. Production of high quality formulated feed needs to be maintained to ensure high yields and large sized fish within short period of time. Although fishmeal is the most preferred protein source in formulated feed, the availability and the increasing price of fishmeal worldwide become more concern compared to its benefits. Thus, an alternative to this common protein source in the making of commercial fish feed is gaining interest among researchers. The replacer or substitute ingredients that could potentially be used are protein sources from aquatic plants, legumes, cereal by-products and animal by-products. Interestingly, the most accessible and inexpensive food component in aquaculture is plant protein sources such as microalgae (Sirakov et al. 2015).

According to FAO (2019), tilapia can be fed with high percentage of plant proteins. However, most of these alternative ingredients are deficient of some nutrients and hence require a combination with other feedstuffs. FAO (2019) also mentioned that the maximum inclusion limits of each feedstuff that can be used in making tilapia feeds is dependent on several factors such as the life stages of fish, economic factor and availability. Besides, the ingredients used in the formulation of tilapia feeds varies between countries. For example, in Thailand, a common feed formulation of tilapia fish contains 16% fishmeal, 24% peanut meal, 14% soybean meal, 30% rice bran, 15% broken rice, vitamin and minerals 1% (Somsueb 1994). In Malaysia, the commercial tilapia feed ingredients contain 15% fishmeal, 5% meat meal, 20% soybean meal, 10% groundnut meal, 10% rice bran, 15% wheat middling, 15% corn or broken rice or cassava, 4% fish oil or vegetables oil, 2% di-calcium phosphate, 2% vitamin premix and 2% mineral premix (FAO 2019).

The reformulation of diets for tilapia with substitution of plant protein source such as microalgae is an alternative way to maintain the production of feeds. Thus, able to solve the problem of decreasing resources of fish meal to meet the needs of aquaculture industry (Hemaiswarya et al. 2011). The increasing attention on microalgae for aquaculture feed is due to its several positive characteristics and because microalgae are at the base of aquatic food chain where tilapia are adapted to consume. Sarker et al. (2016) reported that the use of microalgae *Schizochytrium* sp. improved the feed utilization efficiency, weight gain and fatty acid profiles in tilapia when the microalgae are substituted in its diet. Additionally, this formulated diet also provides high quality and a supplement of long-chain polyunsaturated fatty acids (PUFA) in tilapia fillets (Sarker et al. 2016). The growth performance of tilapia fed on formulated diets with partial substitution of microalgae *Chlorella* spp. and *Scenedesmus* spp. also increased with the increasing replacement of both microalgae species from 0 to 50% microalgae (Bawdy et al. 2008). The researchers also added that the feeding efficiency of tilapia increase with the increasing of microalgae content up to 50%. Dawah et al. (2002) found that the food conversion ratio and protein efficiency ratio were better when the fish were fed with formulated diets containing 10 and 20% of dried microalgae. According to Zeinhom (2004), the substitution of microalgae in fish diet significantly increased the growth performance, and body weight gained on tilapia increased linearly with the increasing level of microalgae in fish diets at levels up to 20%.

5.6 Conclusion

In conclusion, the use of microalgae in fish feed as a fishmeal or other feed ingredients' replacement is not uncommon in aquaculture industry. Due to the high protein composition and other significant macromolecules, microalgae are seen to be a highly potential to be used in making fish feed commercially. In addition, the high growth rates of microalgae indicate that this organism is a potential source of protein substitution in fish feedstock. The high nutritional contents such as carbohydrates, proteins, antioxidants, polyunsaturated fatty acids, pigments minerals and vitamins of microalgae have helped to maintain the good quality of fish production specifically for tilapia as it is the most popular food fish cultured worldwide. The formulated fish feed using microalgae as an alternative for protein is seen able to sustain the food chain and secure the security of food sources in the future.

Acknowledgements The authors also wish to thank The Ministry of Energy, Science, Technology, Environment and Climate Change (MESTECC) under E-Science Fund (02-01-13-SF0135), The Ministry of Education Malaysia under Fundamental Research Grant Scheme (FRGS Vote No. K101) and Universiti Tun Hussien Onn Malaysia under Geran Penyelidikan Pasca Siswazah (GPPS Vote No. H054) for supporting this research.

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Chapter 6
Microbial Fuel Cells: A Green and Alternative Source for Bioenergy Production

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Abstract Microbial fuel cell (MFC) represents one of the green technologies for the production of bioenergy. MFCs using microalgae produce bioenergy by converting solar energy into electrical energy as a function of metabolic and anabolic pathways of the cells. In the MFCs with bacteria, bioenergy is generated as a result of the organic substrate oxidation. MFCs have received high attention from researchers in the last years due to the simplicity of the process, the absence in toxic by-products, and low requirements for the algae growth. Many studies have been conducted on MFC and investigated the factors affecting the MFC performance. In the current chapter, the performance of MFC in producing bioenergy as well as the factors which influence the efficacy of MFCs is discussed. It appears that the main factors affecting MFC's performance include bacterial and algae species, pH, temperature, salinity, substrate, mechanism of electron transfer in an anodic chamber, electrodes materials, surface area, and electron acceptor in a cathodic chamber. These factors are becoming more influential and might lead to overproduction of bioenergy when they are optimized using response surface methodology (RSM).

Keywords Bioenergy · Factors · Optimization · Anode · Cathode

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A. A. S. Al-Gheethi et al. (eds.), *Prospects of Fresh Market Wastes Management in Developing Countries*, Water Science and Technology Library 92,
https://doi.org/10.1007/978-3-030-42641-5_6

6.1 Introduction

The over increasing in the population, industrial, and agriculture activities associate with the need for more energy. The traditional resource of the energy such as fossil fuels is contributing to the environmental pollution. Many of the adverse effects on the environment have been reported in literature since several years ago as a result of overutilization of fossil fuels. Therefore, repair of the damage in the environment is conducted with the long-term solution (IPCC 2014). More attention has been given by researchers to find alternative resources for the energy. Microalgae–microbial fuel cells (MMFCs) is one of the best alternative sources for the energy because algae are photocatalytic organisms with very low nutrient requirements; therefore, algae has a dual role for treatment of wastewater contaminated with nutrients and produce the bioenergy as biohydrogen, biodiesel, and bioethanol as well as biomass yield. Figure 6.1 shows the medical and environmental applications of algae.

Algae are among several photosynthetic organisms which have the ability to convert the solar light into biochemical energy (Mohan et al. 2011; Jais et al. 2017). Several types of wastewater have been investigated as potential substrates for inducing microalgae and bacterial growth in the MMFCs (Pandey et al. 2016; Pahazri et al. 2016; Al-Gheethi et al. 2019). MMFCs with bacteria and MMFCs with algae are quite different. In MFCs with bacteria, the chemical energy available in the chemical bonds of the organic compounds is converted to bioelectrical energy through catalytic reactions of the bacterial cells under anaerobic conditions. Meanwhile, in MMFCs with algae, the bioelectricity is generated as a result of converting the solar light during photocatalysis process. The MFCs consist of two chambers, namely, the anode and cathode chambers. In many cases, bacterial cells are used as anode which release the electron as a result of oxidation process while microalgae are used as cathode which received the electron to be used in the reduction process.

The current chapter aimed to highlight the potential of algae in the MMFCs for production of bioenergy and factors affecting the bioenergy production process.

6.2 Microbial Fuel Cells (MFCs)

Microalgae fuel cells (MFCs) are a device used to convert solar energy into electrical energy as a function of metabolic and anabolic pathways of the algae cells (Lee and Huang 2013). The MFCs are different from the direct methanol fuel cells and other non-biotic fuel systems. The main differences include the complexity of the biotic system with addition of organisms which are responsible for inducing the electrochemical reactions. Moreover, the biological system for the production of bioenergy is more flexible since it can work within a range of temperature (15–45 °C). pH also can contribute efficiently in the treatment of the wastewater (Santoro et al. 2017). The process has received high attention from researchers over the years due to the simplicity of the process, absence of toxic by-products, and low requirements for

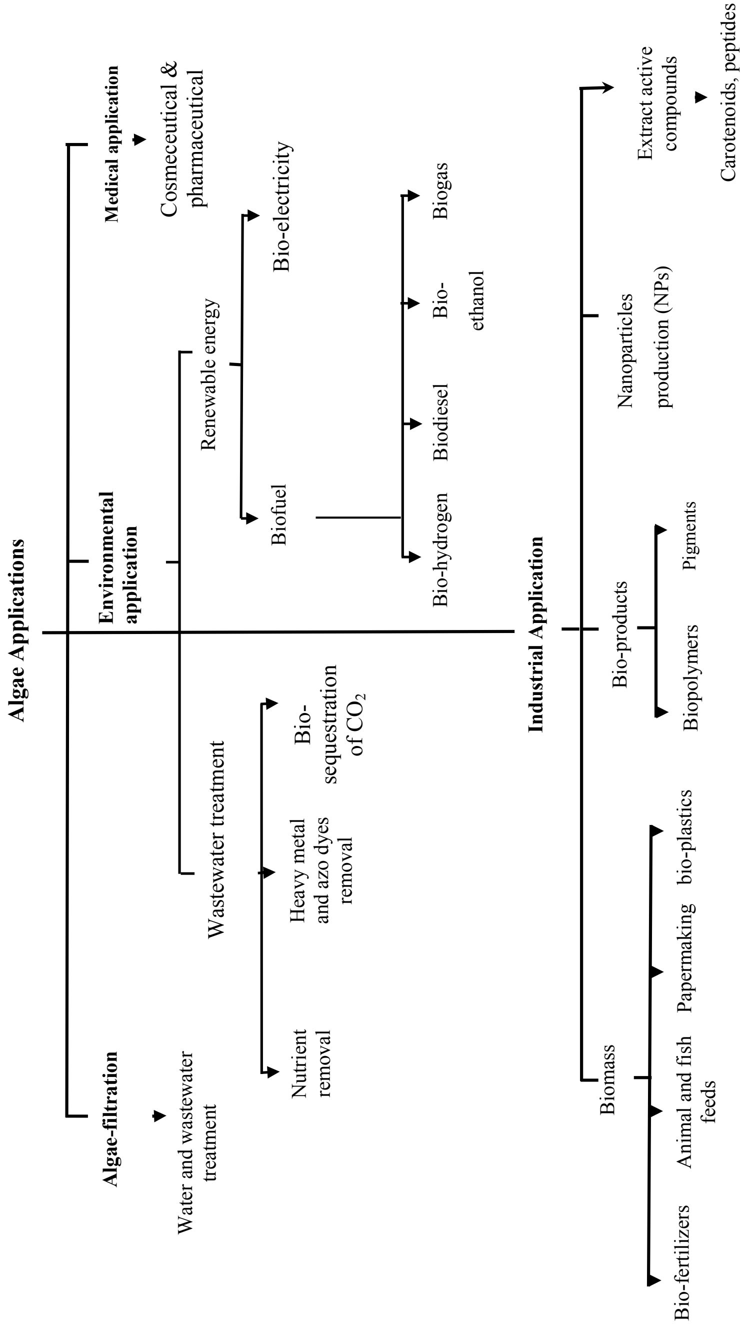

Fig. 6.1 Algae applications in environment and industry

the algae growth. Moreover, the algae biomass generated during the cultivation has several applications as a fuel production and pharmaceutical product. Another advantage of the green technology of algae is the high potential for treating wastewater and removing nutrients, organic pollutants, and heavy metals. In terms of bioelectricity generated from the algae, it depends on the algae activity and growth, such as metabolic pathways and photosynthesis process which associate with the release of oxygen through metabolic pathways and photosynthesis of the microalgae. In more critical details, the light wavelength and period are playing vital role in the amount of bioelectricity production (Luo et al. 2017; Saratale et al. 2017). Several algae species have been used in the MFC components as cathode and anode. However, in many studies, the algae are used as cathodes, *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Spirulina platensis*, *Pseudokirchneriella subcapitata,* and *Anabaena* sp. are among the algae reported in literature (Strik et al. 2009; Zhou et al. 2012; Fu et al. 2009; Xiao et al. 2012; Pandit et al. 2012).

Bazdar et al. (2018) used *Chlorella vulgaris* to develop a photosynthetic microalgae microbial fuel cell (PMMFC) for production of bioelectricity and biomass as well as for treating wastewater as a response for different light intensities (3500–10,000 lx) and periods (24/00, 12/12, 16/8 h dark/light). The study revealed that 126 mW m^{-3} of electricity was produced, while 78, 5.47% of the Coulombic efficiency and COD removal were recorded. In contrast, the biomass yield is correlated with the light intensity up to 10,000 lx. However, authors in literature mentioned that the dark period is required to maintain the health of algae cells. Therefore, in many studies, the algae cells used for bioelectricity are incubated under 16/8 h light/dark cycle (Wu et al. 2013).

Several studies have been designed and developed for harvesting of bioenergy from photocatalysis process of algae. Subhash et al. (2013) developed a single-chambered autotrophic photo-bioelectrocatalytic fuel cells (PhFCOX). Nafion-117 has been used as a proton exchange membrane (PEM) and sandwiched between the anode which was totally immersed in the wastewater samples, where cathode was exposed to the air at the top and immersed in the wastewater at the bottom. The biocatalyst used in the study was algae consortium inoculated into the PhF_{COX} with wastewater and incubated at ambient temperature (12 h light/12 h/dark). The pH was adjusted to pH 7. The electricity activity was assessed in terms of voltage, current, and electron discharges. The study revealed that the maximum voltage generated reached up to 38 mV and 0.1 mA after 120 h. Gouveia et al. (2014) investigated the production of bioelectricity in MFCs via *Chlorella vulgaris* as a cathode and bacterial consortium as an anode. The study revealed that the production of bioelectricity associated with the light intensity ranged from 26 to 96 lE/(m^2 s), and the maximum production was 62.7 mW/m^2 recorded with 96 lE/(m^2 s) of the light intensity.

In many studies, the microalgae–microbial fuel cell system combined microalgae and bacteria. Huarachi-Olivera et al. (2018) suggested that microalgae–microbial fuel cell using *C. vulgaris* and bacterial community exhibited a simultaneous efficiency in the production of bioelectricity and bioremediation processes. The MFCs produced bioenergy ranging from 23.17 to 327.67 mW/m^2 on 32 d. Therefore, the MFCs represent an important source of bioenergy.

6.3 Factors Affecting Bioenergy from MMFCs

Several factors are contributing effectively to the MMFC's efficiency and production of bioenergy. The most common factors include substrate, bacterial and algae species, metabolism activity of the organism, mechanism of electron transfer in an anodic chamber and electrodes materials and surface area. Besides, factors such as pH, temperature, salinity, and electron acceptor in a cathodic chamber are considered as the most important factors in the operating process and they affect directly on the algal and bacterial growth and activity, and thus on the MMFC's performance (Aghababaie et al. 2015).

6.3.1 Effect of Substrate

In many studies, the wastewater is used as a substrate for bacterial and algae growth due to the high contents of nutrients and organic compounds which support the organism growth and acts as a source for electrons required for generating the bioenergy. Several wastewater such as tannery, slaughterhouse, rice mill, swine, cassava mill, dairy, winery, and molasses wastewater have been used as a substrate for bioenergy production by MFCs (Min et al. 2005; Zhang et al. 2009; Kaewkannetra et al. 2011; Mshoperi et al. 2011; Mardanpour et al. 2012; Katuri et al. 2012; Mathuriya 2013; Sciarria et al. 2015). However, fresh market wastewater is the best medium for bacterial and algae growth and also for handling. This is because the fresh market wastewater has high contents of nutrients required for algae growth and rich with organic materials required for bacterial growth and honors for electrons besides having low suspended solids (SS) in comparison to the sewage wastewater. The low concentrations of SS might also support the algae growth because the sunlight can penetrate the wastewater and provide the light required for the anabolism and metabolism activity in the algae cells.

Velasquez-Orta et al. (2011) investigated the efficiency of brewery, bakery, dairy, and paper industrial wastewater in producing bioenergy by MFCs. The study revealed that MFCs fed with paper wastewater generated the highest current density (125 ± 2 mA/m^2), while dairy wastewater produced 25 ± 1 mA/m^2. In contrast, the brewery and bakery wastewaters produced only 10 ± 1 mA/m^2. The authors claimed that the current production was independent of substrate degradability, while the microbial composition of anodic biofilms differed based on the wastewater. The MFC anodes with paper wastewater exhibited the redox activity at -134 ± 5 mV. In comparison with the commercial substances, Chae et al. (2009) tested four types of fed substrates including acetate, butyrate, propionate, and glucose in MFCs with anaerobic sludge. The study found that the Coulombic efficiency (CE) was dependent on the substrate, and ranged from 72.3% in the presence of acetate to 15.0% with glucose.

6.3.2 *Effect of Electrode Materials*

Electrode materials represent a crucial role in the generation of bioenergy in many of the studies. Tsai et al. (2009) tested the potential of carbon nanotube (CNT) modified carbon cloth as an electrode in single-chamber MFCs with wastewater as a substrate for the bacterial growth. The study revealed that the CNT coated onto carbon cloth electrode has improved the power density to 65 mW m^{-2} with 67% of the Coulombic efficiency. Lee and Huang (2013) investigated the effect of electrode spacing (ES) with the range of 5.8–19.5 cm and substrate concentrations in terms of COD (mg/L) on the electricity generation in MFCs. The study indicated that the maximum bioenergy output 3 was 0.32 mW/m^2 recorded at ES 5.8 cm and nominal COD (in) 300 mg COD/L. The study concluded that the ES required for improving electricity generation is dependent on the level of COD. Wang et al. (2017) developed SMFCs with comb-type cathode electrodes and carbon cloths. The cathode electrode was studied with three different exposed areas to investigate their effect on the improvement of oxygen transfer. The study revealed that the maximum power density was 3.77×10^{-2} mW/m^2 with 75% of the (M_{A75}) exposed area, while the lowest was recorded in the completely immersed electrode. The study concluded that the exposed area of the cathode electrode plays a critical role in the power performance of SMFCs. The effect of the presence of the cathode microporous layers and the distance between cathode and anode on the performance of MFCs was studied by Yao et al. (2014). The study revealed a correlation between power density and the distance between cathode and anode, the power density has reduced from 973 to 797 mW/m^2 as a result of decrease in the distance from 2 to 1 cm. However, the power generation increased to 955 mW/m^2 with 0 cm distance. The study also revealed that the presence of aerobic bacteria might induce the non-linear correlation between the power production and electrodes' distance.

6.3.3 *Effect of Bacterial and Algae Species and Density*

Both bacterial and algae species and the initial concentrations used in MFCs play important role in the production of bioenergy. Arbianti et al. (2017) investigated the effect of microbial density (0–10%) by MFCs in producing bioenergy. The study revealed that 1% of the bacterial density was generated between 291.1 mV and 66.33 mW m^{-2} with 4.48% of the Coulombic efficiency. The nutrients were used as an effective energy source in MFCs by the bacterial cells as a biocatalyst. Nonetheless, many of the bacterial species have been used in MFCs. Choo et al. (2006) listed these bacteria including *Burkholderia multivorans*, *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Bacteroidetes*, *Actinobacteria*, *Cyanobacteria*, *Spirochaetes*, β-Proteobacteria, and α-Proteobacteria.

Mei et al. (2015) investigated the performance of bacterial density and community from four sources including garden soil (GS), activated sludge (AS), river sediment

(RS), and wastewater (WW) in MFCs. The study recorded a different power generation with different bacterial densities, while the bacterial community from the RS generated maximum power density (744.8 mW m^{-2}), followed by bacterial from AS, GS, and WW. Based on the principal component analysis (PCA), the microbes in MFC-AS and MFC-GS were closely clustered but separated from MFC-WW to MFC-RS. The majority of microbes in RS was *Azoarcus* (45.20%), while *Flavobacterium* (14.18%) in AS, *Geobacter* (14.40%) in GS, and *Azovibrio* (11.11%) in WW. It can be concluded that the initial concentrations of bacteria and the source play important role in the amount of bioenergy generated in MFCs.

Velasquez-Orta et al. (2009) compared bioelectricity production between *Chlorella vulgaris* and *Ulva lactuca*. The study revealed that *C. vulgaris* was more efficient in producing energy (2.5 kWh/kg) compared to *U. lactuca*. The maximum power densities obtained by *C. vulgaris* were 0.98 W/m/(277 W/m), while were 0.76 W/m/(215 W/m) by *U. lactuca* in single and multiple cycle methods, respectively. These findings confirmed that the production of bioenergy is dependent on the algae species which have different metabolic activities.

6.3.4 Effect of Environmental Conditions

The environmental factors affecting microbial fuel cell efficiency are similar to the factors affecting microbial growth such as pH, temperature, ionic strength, light, and salt. Factors which improve the microbial growth also improve the MFC performance. Salt has negative effect on the microbial growth; however, higher salinity and ionic strength associate with the increasing conductivity of substrate and therefore enhance the MFC performance (Aghababaie et al. 2015).

The effect of different light intensities on bioelectricity in a photosynthetic alga microbial fuel cell (PAMFC) using *Chlorella vulgaris* was investigated by Gouveia et al. (2014). In the study, *C. vulgaris* was used as a cathode compartment, while the bacterial consortium was used in the anode. Two different light intensities were investigated. The results revealed that the highest bioelectricity production was 62.7 mW/m^2 with 96 μE/(m^2 s) light intensity. However, the increase of the light intensity from 26 to 96 μE/(m^2 s) was associated with the increase of bioelectricity production by sixfolds.

Larrosa-Guerrero et al. (2010) studied the effect of temperature between 4 and 35 °C on the performance of MFCs with brewery wastewater as a substrate. The study revealed that the temperature plays a crucial role in the electricity production given the maximum power of 15.1 mW m^3 reactor at 4 °C to 174.0 mW m^3 reactor at 35 °C. Liu et al. (2005) tested the energy recovery, power density, Coulombic efficiency, and electrode potential of MFCs as a function of electrode spacing, solution ionic strength, temperature, and composition. The study revealed that power output increased from 720 to 1330 mW/m^2 with the increase of ionic strength from 100 to 400 mM, decreasing the distance between the anode and cathode (4–2 cm). These might occur as a result of decrease in the internal resistance. Moreover, the power output was reduced by 9% due to the decrease in the temperature from 32 to 20 °C.

The studies conducted on the production of bioenergy as a response to different pH values in the MFCs indicated that the optimal initial pH is between 8 and 10. In MFCs, the microbial process in the anodic preferred a neutral pH and decreases at acidic or alkaline pH. Meanwhile, in the cathode, the biological reaction is high at alkaline pH (He et al. 2008).

6.4 Optimization of Microbial Fuel Cells

Optimization is one of the most important steps that must be taken to obtain a maximum and high-quality bioenergy production by MFCs. Besides that, optimization of MFCs is also conducted to reduce the cost of operating process. Many software programs have been used for the optimization process. However, response surface methodology (RSM) based on central composite design (CCD) is the most common method used due to the ability of this method to provide the best operating parameters required for high production of bioenergy with minimum experimental runs. The optimization process is dependent on the selection of independent factors such as pH, temperature, microbial concentrations, and incubation period which have direct and indirect effects on the performance of MFCs and show interaction that can guide in increasing the overall performance of MFCs. For instance, Madani et al. (2015) found the best operating parameters for MFC's performance from the interaction between pH and buffer concentration. The maximum power generated was 461 mW m^{-2} (at pH of 6.3 and buffer concentration of 82 mM).

Hosseinpour et al. (2014) used RSM to optimize the power generation by MFCs as a response for three independent factors including buffer concentration, pH, and ionic strength. The study revealed that the highest power density was increased by 17% at 0.11 M buffer concentration, pH 6.75, and 4.69 mM ionic strength of cathode chamber. The maximum Coulombic efficiency (3%) in MFCs with power density of 1097 mW/m^3 was reported in the study by Fang et al. (2013). This was observed at 102 mM ionic concentration, pH 7.75, and with 48.4 mg/L of nitrogen at 30.6 °C.

6.5 Conclusion

Based on this review, it can be concluded that MFC is one of the best alternative methods to getting a green energy. However, many factors should be considered, in which among them the microbial strains, environmental conditions, cathode and anode types, and substrates. Moreover, the optimization of these independent factors might contribute effectively in the high production of bioenergy at lower operating cost.

Acknowledgements The authors would like to thank Research Management Centre (RMC), UTHM for providing GERAN KONTRAK UTHM (H377) as a financial support for this research project.

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Chapter 7
Reduction of Seafood Processing Wastewater Using Technologies Enhanced by Swim–Bed Technology

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Abstract The increasing growth of the seafood processing industries considerably requires more industrial process activities and water consumption. It is estimated that approximately 10–40 m^3 of wastewater is generated from those industries for processing one-tonne of raw materials. Due to limitations and regulations in natural resources utilization, a suitable and systematic wastewater treatment plant is very important to meet rigorous discharge standards. As a result of food waste biodegradability, the biological treatment and some extent of swim-bed technology, including a novel acryl-fibre (biofilm) material might be used effectively to meet the effluent discharge criteria. This chapter aims to develop understanding on current problems and production of the seafood wastewater regarding treatment efficiency and methods of treatment.

Keywords Seafood processing industries · High-strength wastewater · Treatment methods · Biological treatment

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A. A. S. Al-Gheethi et al. (eds.), *Prospects of Fresh Market Wastes Management in Developing Countries*, Water Science and Technology Library 92,
https://doi.org/10.1007/978-3-030-42641-5_7

7.1 Introduction

Disposal of wastewater is one of the important issues in many countries due to the increase of contamination level in the natural sources such as soil, surface water and groundwater (Al-Gheethi et al. 2013, 2014). Biological wastewater treatment processes (BWWTP) were developed for faster treatment of contaminated water sources. Seafood processing is a growing industry for many countries with coastline like Malaysia. The seafood industry represents significant parts of economic resources for exports, which is currently ranked in the top 10 export value in Malaysia (Porntip et al. 2006). Therefore, keen considerations are developed to assist the seafood industries to meet water quality requirements as per country standard (Weerasekara et al. 2016).

Many types of seafood are immediately treated based on seasonality, frequently at the same location of plant. Saltwater fish (tuna, sardines), mollusks (oysters, clams), crustaceans (crabs and lobster) and others such as shrimp and octopus are immediately treated based on seasonality. Therefore, a suitable and rational treatment plant design is needed to meet the recent regulation. Hence, the first step in designing a wastewater treatment plant in seafood process industry depends largely on the nature of the used water generation, which varies on the working hour, day, week and season. The second step is the sampling should be based on 24 h of normal and peak effluents discharge basis. This is because the same peak might carry the same load of contaminants, i.e. biochemical oxygen demand (BOD), nitrogen, fats, oil and greases (FOG) on each peak flow. The best location of sampling is the point before the effluent being discharged to the receiving water body (Chowdhury et al. 2010). However, it is difficult to understand the severity of the problem created by seafood wastewater streams because it depends on the strength of the effluent, the rate of discharge, and the actual capacity of the receiving water body. Regulations on wastewater are becoming more and more complicated since it involved a larger demand for suitable design and higher treatment efficiency for such effluent. However, key pollution parameters should be taken into account for design consideration when determining the characteristics of wastewater and evaluating the efficiency of wastewater treatment system (Ferjani 2005; Chowdhury et al. 2010).

A new technology including a novel acryl-fibre biofringe (BF) material is an exceptionally effective aerobic treatment for wastewater. The BF material provides biomass attachment on the flexible material within a fixed position matrix. Through this approach, fibre matrix induced swimming motion because of the wastewater flow, which enhances nutrients transfer to the attached biofilm. This motion increases the detachment of excess biomass, in the form that can be easily settled and removed (Baker et al. 2012; Baker 2016). This chapter aims to develop the understanding on current problems and production of the seafood wastewater regarding treatment efficiency and methods of treatment.

7.2 Solid and Liquid Waste Management

Seafood processing industries generate wastewater because there are many steps of operation that involved in each stage of recycling and reclamation. The disposed wastewater should be environmentally acceptable and in compliance with the regulations by global law which are applicable in many countries (Vikneswara et al. 2019). Contamination of soil, ground and surface waters due to wastewater should be prevented, and necessary measures are required for proper disposal of wastes such as hazardous material, process residues, solvents, oils and sludge. The disposal of seafood waste to marine environment (i.e. river, sea, etc.) are advised to perform sieve analysis through a 5 mm sieve before the waste can be discharged. The location for disposal of waste is also crucial to prevent a build-up of solid formation (Goel et al. 2005).

7.2.1 Strategy and Plans for Liquid Waste Management

Many steps should be considered to achieve the quality in the satisfactory level with existing facilities. If no initial treatment been carried out to meet the standard discharge criteria, then the receiving environment will be adversely affected due to high load of contaminants. Planning for waste management is largely dependent on the financial and facilities for the upgrading situation (Aziz et al. 2009). This means that it would upgrade the level of the raw sewage discharge before going into receiving water bodies (rivers, seas and oceans) channelled by primary and secondary treatment, as shown in Fig. 7.1. Liquid waste management plans (LWMP) are used to find the schedule to upgrade secondary treatment. Therefore, LWMP include: (i) public consultation, (ii) schedule to upgrade all liquid waste discharges to water bodies, (iii) a schedule and means to address all municipal liquid waste (Roy and Raj 2010; Wang et al. 2016).

Liquid waste management includes sewage treatment, wastewater treatment, and biochemical and chemical treatment (Banashri and Annachhatre 2007). It applies different ways of treatment for rural and urban areas, in developed and developing countries. The value of treated wastewater is usually worth when the wastewater can be reused. There are different methods through which wastewater is reused, and are named as physical reprocessing and biological reprocessing (Demirbas 2011). Wet biomass like sewage sludge and some industrial wastewater can be converted to produce fertilizer and fuel. Since biomass is easy to be converted into liquid fuel, it can then be used for transportation fuel in the future for trains, cars, and buses (Demirbas 2011).

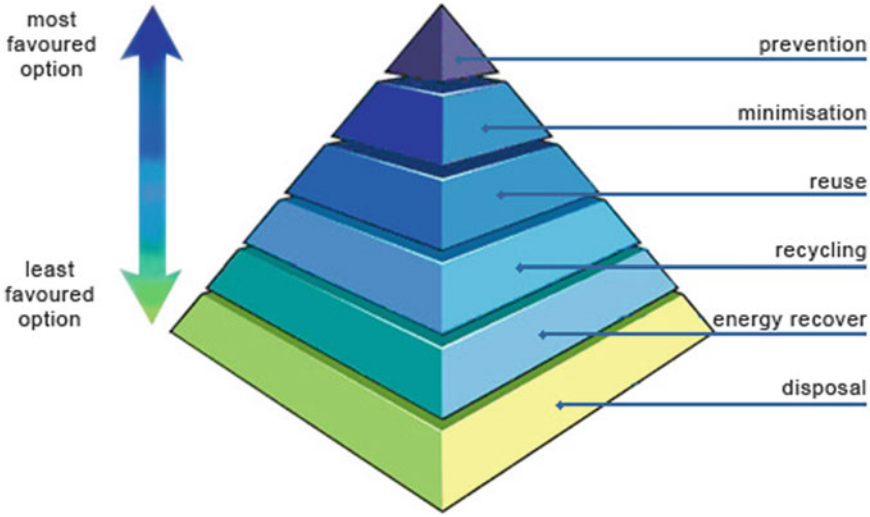

Fig. 7.1 The waste hierarchy

7.2.2 Seafood Processing Industries

Seafood processing industries produce large quantities of organic waste, which remained in the generated wastewater. Residual of waste includes food remnants from houses and commercial establishments such as restaurants, and cafes. The total generations of food wastewater in terms of quantities are approximately half, with the other half coming from municipal solid waste streams. The BOD of the food processing industries wastewater depends on the type of industry and raw materials used US EPA, (2005). The dairy industry produces wastewater with BOD ranging from approximately 1000 mg/L (milk or cheese plant wastewater) to 35,000 mg/L (whey wastewater). The meat industry wastewater had a BOD ranging from 400 to 11,000 mg/L. The seafood, edible oil, confectionary food and brewery processing industries similarly produce a few hundred to thousand mg/L range of BOD. Such types of wastewater are usually entering a common stream with municipal wastewater. However, in most cases, food caste processors enquired extra charges due to high level of BOD over than sewage wastewater.

According to Afonso and Borquez (2002), food processing wastewater contains substances such as carbohydrates, fats, oils and proteins. The release of the wastewater from food industries to the environment without proper treatment might incur negative effects on the environment. Proteins as a source of carbon in the wastewater are precious resource for energy production. The use of biological methods such as biofringe and anaerobic digestion have been investigated for treatment of wastewater from seafood processing industry. Biofringe and anaerobic digestion are suitable options for a very high BOD level, and not for wastewater with lower BOD level.

Alternative emerging technologies such as biofringe with combined fibres offer a potential solution for wastewater streams (Weerasekara et al. 2016).

7.2.3 *Characteristics of Seafood Industry Wastewater*

The main concern of seafood processing wastewater is their characteristics including contaminant parameters, sources of process, and types of resource used. The characteristics of seafood processing wastewater are equivalent to sewage wastewater including parameters such as physicochemical properties, organic matter, nitrogen and phosphorus contents. Important indicators for pollutant constituents of wastewater include BOD_5, chemical oxygen demand (COD), total suspended solids (TSS), oil & grease and heavy metals. As in most industrial wastewater, the contaminants present in seafood processing wastewater are undefined mixture of substances, mostly organics in nature. It is impossible to do a detailed analysis for each element present; therefore, overall measurement on the degree of pollution is sufficed (US EPA 2003; Chowdhury et al. 2010).

7.2.4 *Process of Seafood Production and Wastewater Generation*

There are many groups of products from seafood industries based on raw material types (i.e. fresh/frozen) and value-added (degree of processing and value content). Figure 7.2 shows some seafood product categories in seafood processing industry (Sohsalam et al. 2008; Rodriguez et al. 2011). Seafood processing units produce wastewater, which results from activities of washing and cutting of raw materials, defrosting, gutting, scaling, portioning and filling of fish. Therefore, the wastewater contains large quantities of organic matter, nitrogen and phosphate, and heavy metals from freezing and canning processes. The effluent stream from canning operations includes cleaning of cans, brines and oil from filing operation, spilling of sauces and precooking condensate. When the effluent streams are discharged without treatment into river or sea, the pollutant will cause eutrophication and oxygen depletion. Figure 7.3 shows the generation of wastewater from seafood processing industries (MetCalf and Eddy 2004; Sohsalam et al. 2008; Rajab et al. 2017).

Seafood processing wastewater consists of various types of wastes such as blood, offal products, viscera, fins, fish heads, shells, skins, and meat fines. The main operations in the seafood industry include the process of receiving the product, boat unloading, sorting, weighing, preparation (cutting, scaling, filleting, skinning, gut), inspection and trimming, and processing of products such as marinades, and further processing (canning and packaging), packaging, and dispatching. However, most of the organic materials in the wastewater such as blood and gut materials come from

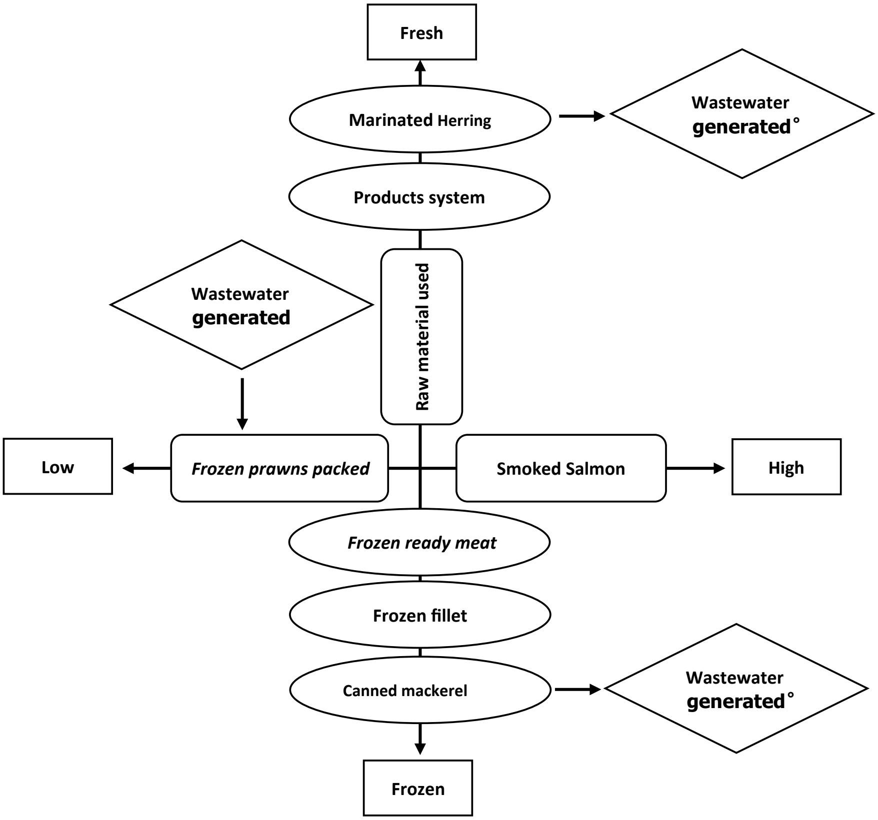

Fig. 7.2 Seafood products in a seafood industry and product wastewater

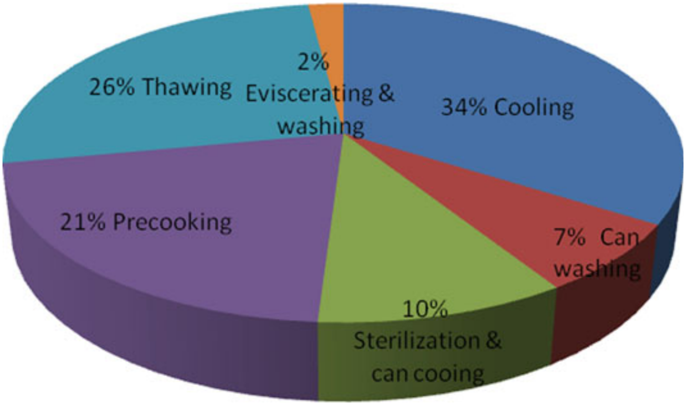

Fig. 7.3 Different activities that generate wastewater

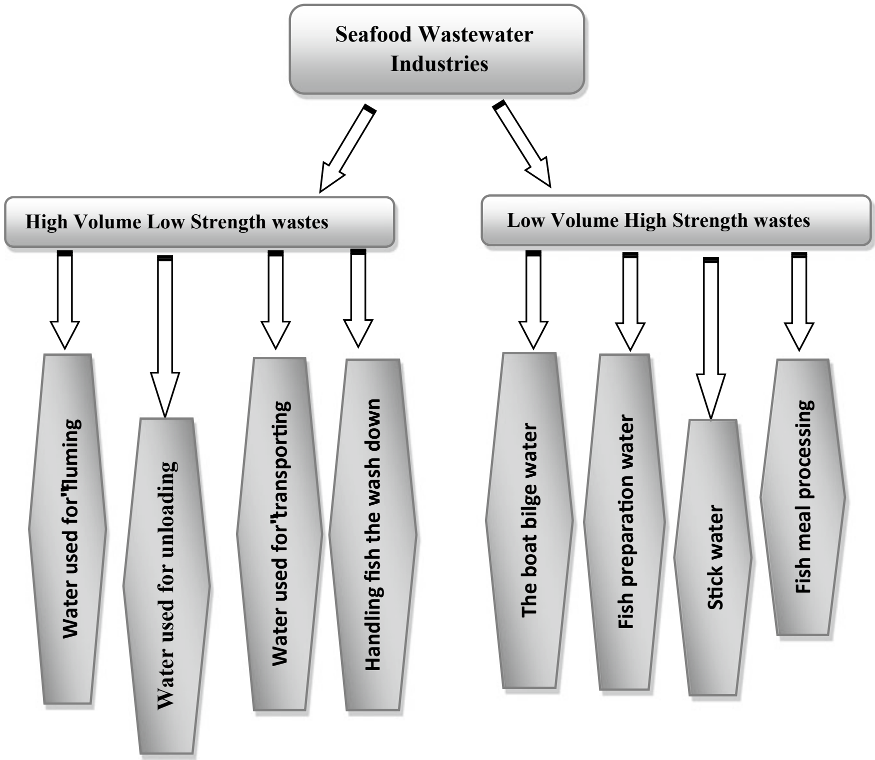

Fig. 7.4 Category of wastewater from seafood processing industry

the butchering process, which are produced in the majority of these processes. Generation of wastewater from seafood processing industries is presented in Fig. 7.4 (Chowdhury et al. 2010; Rajakumar et al. 2011).

7.3 Seafood Processing Wastewater Treatment Options

Sampling and analysis are very important steps in designing a wastewater treatment system. However, treatment of seafood processing wastewater largely depends on the generation time. Sampling of wastewater includes all types of operations for processing seafood; therefore, samples collection must consider the generation time. The best sampling location is before the discharge point to receiving water body. Contaminants such as TSS, FOG, COD, BOD, nutrients are very important to characterize and categorize the generated wastewater. It is always a good idea to perform jar tests to determine the type of chemicals' treatment, such as pH adjustment (acid and base), coagulants, flocculants, and attach growth. A gradual approach to wastewater treatment usually gained better results in the most economical way. The primary treatment removes suspended solids, colloidal materials, large particle, and settleable solids. Solids and colloids in the treatment of seafood wastewater should be removed fast using low-cost technologies to avoid dissolution thus having more cost-effective means (Chowdhury et al. 2010; Oyanedel-Craver et al. 2009; Sarti et al. 2016).

7.3.1 Primary Treatment

Seafood processing wastewater contains large amounts of insoluble suspended solids, which must be removed from waste effluents through chemical and physical treatments. Before the implementation of other treatments, primary treatment to remove waste components is desirable because a major consideration in designing a treatment system is that the solids should be removed as quickly as possible. Delaying the treatment will cause difficulty in solids removal or by-product recovery. This is because the longer detention time will increase BOD_5 and COD level making it hard for the aquatic life to survive and react with the pollutants. Like other primary treatment processes, seafood processing wastewater uses screening, sedimentation, flow equalization, and dissolved air flotation as primary treatment. Those unit operations will generally remove up to 85% TSS, and reduced 65% BOD_5 and COD from wastewater (Chowdhury et al. 2010; ODNR et al. 2010).

Literature revealed that flow through screen is the simplest way, with openings of approximately 1 mm. Screening is achieved by the removal of relatively large solids ($\geq$0.7 mm). The use of this treatment approach in food processing plants made it possible to reduce the amount of solid materials in the discharge effluents quickly. Sometimes a scrapping mechanism is very important to minimize the clogging problem in this process as shown in Fig. 7.5. There are two types of screening methods used for seafood processing wastewater, first tangential screening and second rotary drum screening. Tangential screens are static, with fewer slopes to clogging due to their flow characteristics. The solids removal rates vary from 40 to 75%. Rotary

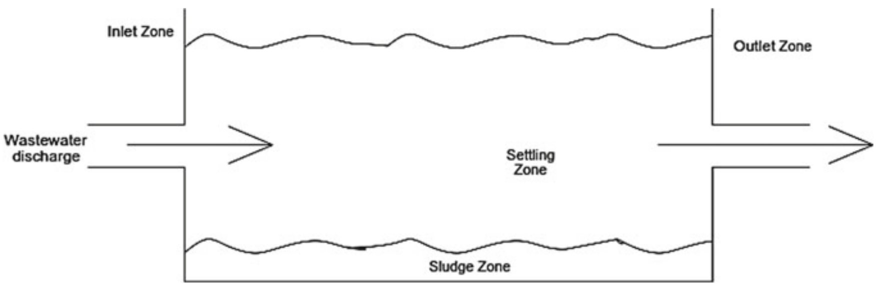

Fig. 7.5 Schematic diagram of discrete settling

drum screens are mechanically operated and complex in nature which consist of a cylinder for rotating along its axis, and the effluent enters through an opening at one end. However, it causes the breakdown of the solids, making it more difficult to separate; high-intensity of waste streams should be minimized before screening or even settling (Metcale and Eddy 2004; Chan et al. 2009; Mekonnen and Hoekstra 2015).

Sedimentation is used widely in the wastewater treatment industry. Sedimentation in the simplest form of removing the heavier particles from the water through deposition due to mass of particles in the bottom of the tank or basin. In general, sedimentation separates solids from water body using gravity settling of the heavier solid particles.

This operation is used in the primary and also in secondary treatment to separate the generated solids in biological process units such as activated sludge or trickling filters. Sedimentation proceeds in many ways such as discrete settling, flocculent settling, and zone settling based on the properties of the present solids. The average removal efficiencies for BOD_5 was reported to be 91–99% by using sedimentation. In fish-processing wastewater, the sedimentation removed 52–90% for SS and 72–92% for TN and 72–77% for TP performances, found at 5 days HRT (Sohsalam et al. 2008). A schematic diagram for discrete settling approach is shown in Fig. 7.5 (Baker et al. 2012; Baker 2016).

Flow equalization is crucial in reducing the hydraulic flow loading of the waste stream. Equalization units consist of a flat tank and pumping equipment to reduce the fluctuations of the waste streams. The equalizing tank increases flow loading and makes flow rate to a uniform discharge over a 24-h day. Removal percentage of BOD_5 from 50 to 55% was achieved in equalization tank of seafood wastewater treatment plant by using fluctuations system and removed 40% of oil and grease and 50% of suspended solids (Goel et al. 2005; Hasar et al. 2009). Flow equalization is normally used near the head end of the effluent discharge to the receiving water bodies (Li et al. 2018).

Seafood processing wastewater contains large amount of oil and grease, which will vary depending on the process used, the type of treatment, and operational procedure. Gravitational separation is used to remove oil and grease. There are two ways to breakdown emulsion: pH adjustment and heat. However, heat may not be economical unless there is excess steam available as shown in Fig. 7.6 (EPA 2003; Kim et al. 2010).

Electrocoagulation can also be applied to treat seafood processing wastewater. Investigation on the effective conditions and several electrode materials showed that aluminium was favoured compared to iron. The best charge loading reported was 1.67–9.95 F/m^3 wastewater with current density of 30–80 A/m^2. The removal percentage of 94% for oil and grease was achieved for the wastewater tested (Chen et al. 2000).

Among effective systems used to remove oil and grease is the dissolved air flotation (DAF) procedure (reference). DAF uses air bubbles to remove suspended matter from wastewater stream. The air bubbles attach themselves to discrete floating particles of oil and grease. The attachment reduces the specific gravity and increases

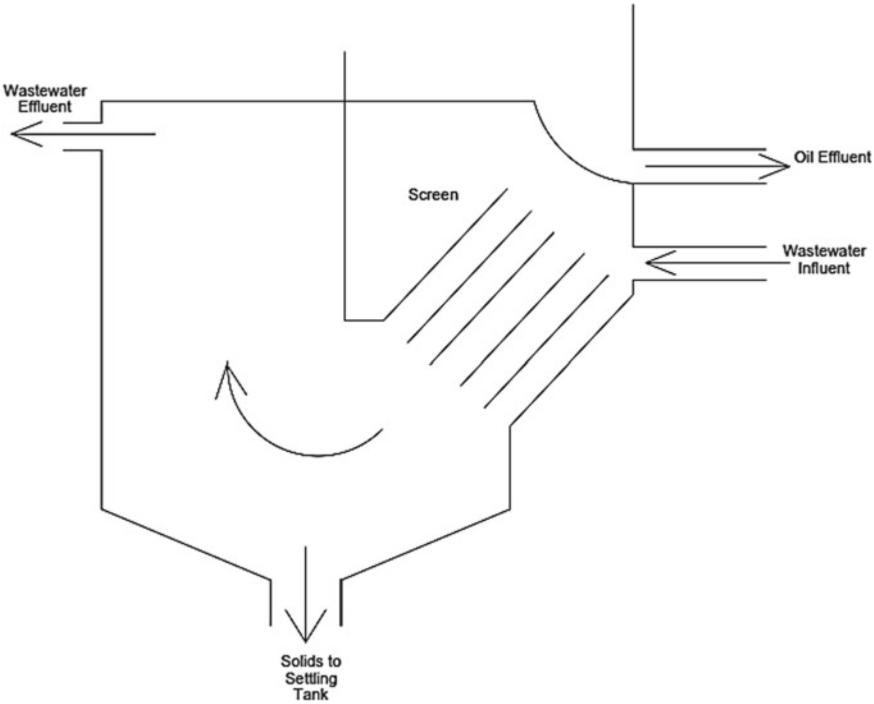

Fig. 7.6 Typical configurations for inclined media separators (Kim et al. 2010)

the vertical rate, causing the particles to be carried towards the upper direction with vertical movement. Oil removal was reported to be 90% by using DAF. In tuna processing wastewater, the DAF removed 80% of oil and grease and 74.8% of suspended solids (Chowdhury et al. 2010; FAO 2017).

Seafood processing industries in Malaysia use biological treatment before discharging effluent to sewer system. Biological treatment involves the use of microorganisms to remove dissolved nutrients from seafood processing wastewater stream. For example, the presence of nitrogen, phosphate and organic materials in the wastewater stream serve as nutrients for the growth of the microorganism under aerobic, anaerobic or facultative conditions. Such conditions use oxygen in different way. Aerobic microorganisms used oxygen for their growth. In contrast, anaerobic microorganisms cannot grow in the presence of oxygen. Facultative microorganisms, on the other hand, can live and maintain growth with or without oxygen through different metabolic processes. Microbes which consume organic material for growth are classified as heterotroph in terms of nutritional point of view (Borole and Hamilton 2010). The process and mechanism of microorganism activities in biological wastewater process is complex and interconnected with substrate and temperature domain. Biological systems convert approximately one-third of colloidal particles from wastewater as organic dissolved and constantly produce and convert the remaining to new cells that are removed by gravity separation settlement. Seafood wastewater consists of very less amount of non-biodegradable portion. In aerobic processes carbon dioxide is released, whereas anaerobic processes release carbon dioxide and methane (Chowdhury et al. 2010; Metcalf and Eddy 2004; Dadi et al. 2018). In biological treatment, the growth can be either suspended growth processes or attached growth processes.

The principle of biological process used for wastewater treatment could be divided into two main categories, namely suspended growth and attached growth or (biofilm) processes. Suspended growth systems are principally aerobic processes with more biomass (Wang et al. 2009; Zhang et al. 2009; Zhang 2009). It enhances the conversion of biodegradable organic materials in wastewater constituents and certain inorganic fraction into cell masses. The available suspended growth systems include complete mix and series flow system with high rate, extended aeration system, contact stabilization and tapered aeration modification system and deep shaft system, and pure oxygen (Vesilind 2003). These processes are used in municipal and industrial wastewater treatment plant under aerobic condition. However, suspended growth anaerobic reactors are used in high organic concentration industrial wastewater and organic sludge treatment (EPA 2003; Metcalf and Eddy 2004; Chan et al. 2010).

This technology is based on the principle that microorganisms grow as microbial layer on a given surface (medium like polyethylene material and plastic), and this surface is exposed to the wastewater for adsorption of organic material and to the atmosphere for oxygen (Bathe et al. 2010). The beds in the installation of fixed-film biological treatment are rectangular shaped while circular beds with rotary distributors are later introduced. An attach growth bioreactor also minimizes the adverse effects of the suspended microorganisms on membrane fouling in submerged membrane bioreactor (Vesilind 2003; Van Den Hende et al. 2014; Bustillo-Lecompte et al. 2016). The main advantages are higher biomass concentration in the reactor tank corresponding to lower wastage of biomass, consortia of aerobic and anoxic metabolic activity within the same biomass ecosystem; lower sensitivity to toxicity and other adverse effects; and upgraded conventional treatment system to existing system at low cost (Loukidou and Zouboulis 2001; Bohdziewicz and Sroka 2005; Chang et al. 2010).

7.3.2 Aerobic Process

Nitrogen and phosphorus are most commonly used as nutrient in seafood processing wastewater; however, oxygen-supplying condition is very important to increase efficiency and successfulness of operation (Wang et al. 2010). The aerobic operations are commonly used in many systems such as activated sludge systems, lagoons, trickling filters, and rotating disc contactors. Describe the following reaction is occurring during the aerobic process:

$$\text{Organic} + \text{O2} \Longrightarrow \text{cells} + CO_2 + H_2O$$

In an activated sludge treatment system, the growth of microorganisms (sludge) occurred through their interaction with organic materials in the wastewater in the presence of excess dissolved oxygen and nutrients such as nitrogen and phosphorus. Microbes convert the organic matters in wastewater to produce carbon dioxide, new cells and energy. Oxygen from free air helps to oxidize the operation. Majority of the activated sludge systems used in seafood processing wastewater industry are extended aeration types; it combines between long aeration time and low organic loadings for 1–2 day detention time. The suspended solids concentration helps treatment in low load, where usually the content of BOD_5 is less than 800 mg/L (Metcalf and Eddy 2004; Baker et al. 2012).

Aerated lagoons, known as small area, are used when land is not available or very expensive (not economically) compared with activated sludge system. Removal percentage from 90 to 95% of BOD_5 was achieved in biological treatment of seafood wastewater treatment plant by using lagoon system. Two types of aerated lagoons are used in seafood processing wastewater treatment: completely mixed lagoons and facultative lagoons (EPA 2003; Baker 2015).

The main type of trickling filter is attaching cell (biofilm) processes. It is different from the activated sludge systems and aerated lagoon systems and used the suspended growth processes (suspended biomass). All biomass is attached to support matter to help biomass for growing. Biomass is the representative of microorganisms available in trickling filters, namely *Alcaligenes*, Flavobacterium, *Streptomyces*, *Nocardia*, *fungi,* and *protozoa,* etc. The important thing in all these processes is that the organic materials which interfere with wastewater are degraded by the microorganisms attached in the supported and used organic material from wastewater. Oxygen is provided in this process to enter the biomass and helps the organic materials to grow biomass layer because proliferation of microorganisms requires oxygen. Lack of oxygen may result in detachment of biomass from support, and they will find another place or spot to grow.

Many design standards are adopted to achieve good operation such as (i) roughing filters are loaded at the rate of 4.8 kg BOD_5/day/m^3 filter media and achieve BOD_5 reductions of 40–50%; (ii) high rate of filters achieve BOD_5 reductions of 40–70% at organic loadings of 0.4–4.8 kg/BOD_5/day/m^3, and (iii) standard rate filters are loaded at 0.08–0.4 kg/BOD_5/day/m^3 and achieved BOD_5 removals greater than 70% (Aziz et al. 2011a, b; Chowdhury et al. 2010; Almandoz et al. 2015).

7.4 Swim–Bed Technology

Swim–bed technology consists of acryl resin fibre carrier, named as biofringe (BF). Swim-bed BF treated high rate organic wastewater stream and proved efficacy through its ability to carry bacteria for longer time and remove the sticking sludge

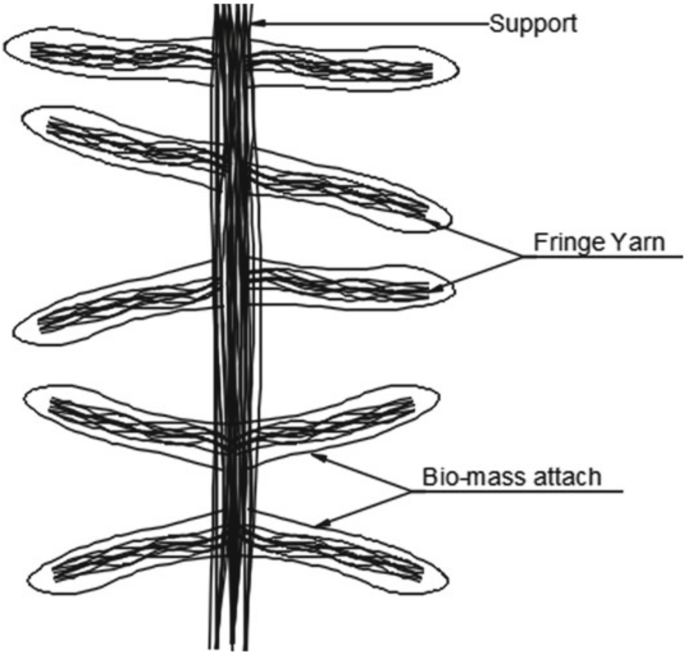

Fig. 7.7 Details of the bio-fringe attachment materials

continually. High levels of microorganisms appear on and inside sticking sludge, which reduces dramatically. The BF fibre involves 3 mm in diameter and 90–100 mm in length of fringe yarns which is attached with support filament as shown in Fig. 7.7. The fringe yarns are fixed symmetrically at equal on the support filament and twisted to give three-dimensional distribution (Cheng 2006; Baker 2016) (Fig. 7.8).

The BF material has rough texture with porous surface. It assists in attaching a large amount of the sludge during treatment. Forms of fringe yarns as a flexible matrix in fixed position are induced from water flow to flex. Swimming motion enhances mass transfer of nutrients to the biofilm. At the same time, swim–bed BF technology provides benefit to both fixed bed and fluidized bed process. Rejection of the head losses and absences of clogging and channelling are unavoidable in fixed bed process. However, BF process continues without depending on hydrodynamic condition and avoids settling or floating of attachment medium or requirement of screens or traps to prevent washout which is not achieved in fluidized bed process (Cheng 2006; Li et al. 2018).

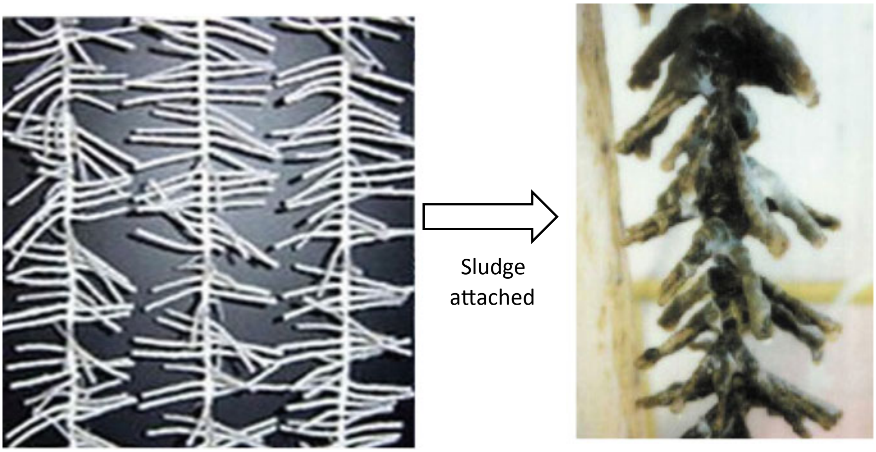

Fig. 7.8 Image of fibre yarns and their arrangement

7.5 Conclusions

The contamination level of seafood processing wastewater depends on many parameters, such as type of operations, type of seafood processed, and the amount of seafood processed. The untreated wastewater might pollute the receiving natural or artificial surface water bodies. Therefore, seafood processing wastewater treatment for all pollutant parameters is necessary before it can be discharged to surface water or groundwater bodies. Seafood processing wastewater consists of high pollutants load, high organic nitrogen, a large part contains food waste which is easily biodegradable such as biochemical oxygen demand (BOD), fat, oil and grease (FOG), and nitrogen content, inorganic salts, and heavy metals. Accumulation of the pollutants is the cause for many diseases to human beings. The biological treatment and BF bioreactor might be another alternative to replace the expensive or inefficient conventional methods. The performance of suspended growth fixed-bed biofringe-reactor with activated sludge has been proven to efficiently remove nitrite, nitrate and phosphorus due to anaerobic and aerobic phase structure; however, the removal performance was not significant for ammonia nitrogen and colour (Wang et al. 2008; Wu et al. 2008; Li et al. 2018). The attached growth concept is designed to minimize the effects of the suspended growth process. Attached growth process enhanced treatment by prolonging contact time with active microorganisms in the reactor. Besides, this process exhibits lower sensitivity to adverse environmental condition. Additionally, sludge reduction was achieved in BF reactor. Nevertheless, choices of the particular treatment mainly follow the wastewater characteristics, existing treatment, and compliance with regulations.

Acknowledgements The authors would like to thank Research Management Centre (RMC), UTHM for providing GERAN KONTRAK UTHM (H377) as a financial support for this research project.

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Chapter 8
Biofilter Aquaponic System for Nutrients Removal from Fresh Market Wastewater

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Abstract Aquaponics is a significant wastewater treatment system which refers to the combination of conventional aquaculture (raising aquatic organism) with hydroponics (cultivating plants in water) in a symbiotic environment. This system has a high ability in removing nutrients compared to conventional methods because it is a natural and environmentally friendly system (aquaponics). The current chapter aimed to review the possible application of aquaponics system to treat fresh market wastewater with the intention to highlight the mechanism of phytoremediation occurs in aquaponic system. The literature revealed that aquaponic system was able to remove nutrients in terms of nitrogen and phosphorus.

Keywords Aquaponics · Biofilter · Nutrient removal · Plant · Fresh market

8.1 Introduction

The expansion of population is associated with the increase of demands for consumable items such as fish, chicken, lamb and cow. This situation increases the number of fresh markets which consequently increase the amount of wastewater generated. Direct discharge of the fresh market wastewater into the environment can negatively affect biodiversity. Therefore, a proper wastewater treatment is needed to avoid environmental degradation such as eutrophication and the spread of waterborne diseases (Haseena et al. 2017). Usually, a fresh market involves slaughtering activities to facilitate customers. Wastewater generated from this activity contains a high amount of organic matter including proteins, blood residues, fat and lard (Apandi et al. 2017; Yaakob et al. 2018). A direct discharge of the untreated slaughtering wastewater to

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A. A. S. Al-Gheethi et al. (eds.), *Prospects of Fresh Market Wastes Management in Developing Countries*, Water Science and Technology Library 92,
https://doi.org/10.1007/978-3-030-42641-5_8

water bodies may lead to eutrophication problems due to the high levels of nitrogen and phosphorus (Yaakob et al. 2018). It was estimated that 90% of all wastewater in developing countries is discharged untreated directly into rivers, lakes or the oceans (Naidoo and Olaniran 2014). Therefore, a treatment for fresh market wastewater shall be taken to prevent high organic loading into the water bodies system.

Many nutrient removal technologies have been applied for fresh market wastewater treatment such as ion exchange process, completely autotrophic nitrogen removal over nitrite (CANON) process and simultaneous nitrification, anammox and denitrification (SNAD) (Rahmani and Mahvi 2006). These approaches are mostly performed for nutrient removal. CANON, for example, has been used to remove ammonium nitrogen and total nitrogen. Based on the studies in the literature, the method can remove up to 93% of ammonium nitrogen and 81% of total nitrogen in wastewater. However, this method requires a long retention time to prevent washout of the suspended nitrifiers from the reactor. Thus, the biological treatment of wastewater using plant need to be highlighted in order to overcome problems related to conventional treatment of nutrient removal technologies in fresh market wastewater. This is due to the capability of the biological treatment method which is considered as environmentally friendly and cheap in terms of maintenance and operating cost. It is also known to be effective for the removal of nutrients and heavy metals for the prevention of environmental pollution (Nandeshwar and Satpute 2014; Al-Gheethi et al. 2015). This chapter aims to review the possible application of aquaponics system to treat fresh market wastewater with the intention to highlight the mechanism of phytoremediation occurs in aquaponics system.

8.2 Interpretation of Fresh Market Wastewater

Fresh market wastewater is a combination of one or more domestic effluents consisting of water from commercial establishments including industrial effluents either as dissolved or suspended matter. The sources of wastewater include washing water (floors cleaning, industrial processing waters, urban runoff). Besides, the main wastewater from fresh market distributed by the blood (including fish gut and others innard organs) from edible animals contain high nutrient and direct discharge to water bodies. The composition of wastewater varies widely. The fresh market wastes water that contains a number of contaminants and pollutants, which include nutrients (nitrogen, phosphorus, potassium); pathogenic microorganisms (viruses, bacteria and protozoa); heavy metals (cadmium, lead, mercury and zinc); organic pollutants (pesticide and herbicide); biodegradable organics (Biochemical Oxygen Demand, BOD and Chemical Oxygen Demand, COD), and micro-pollutants such as medicines and cleaning agents (Al-Gheethi et al. 2016; Jais et al. 2017). Hence, environmental conditions arising from inefficient wastewater management can pose a major threat to human health, wellbeing and economic activity (Noman et al. 2019).

8.3 Aquaponic Biofilter System in Malaysia

The development of aquaculture industry has become a major economic importance worldwide. Aquaculture continues to show increasing production at an average annual growth rate of 6.1% between 2002 and 2012. The production increased from 36.8 million tonnes in 2002 to 66.6 million tonnes in 2012. Major aquaculture producers in 2012 were China (41.1 million tonnes), India (4.2 million tonnes), Vietnam (3.1 million tonnes), Indonesia (3.1 million tonnes), Bangladesh, Norway, Thailand, Chile, Egypt and Myanmar. These producers contributed 88% of the total aquaculture production worldwide. Aquaculture in Malaysia started in the 1920s with multiple carp species reared in ex-mining pools. Since then, apart from the crude oil, palm oil and rubber industries, the aquaculture industry has developed into a profitable and sustainable industry as shown in Fig. 8.1. It was shown that the total production of aquaculture in Malaysia in 2016 was 7,110,600 metric tonnes which are seaweed dominant with total production approximately 3,300,000 metric tonnes. However, the freshwater species showed to increase in total production from 2014 to 2016 by 44%. One of the main factors that contributes to this trend is the current governing policy for the development of aquaculture in Malaysia which is the National Agro-Food Policy (NAFP) 2011–2020 (Witus and Vun 2016). The introduction of this policy will motivate Malaysian to participate in aquaculture industry. As for the aquaculture sector, good practices in conducting aquaculture activities will be compulsory under Aquaculture Industry Zone programme (Witus and Vun 2016).

Freshwater pond culture has been the greatest contributor to local aquaculture production. Witus and Vun (2016) stated that the aquaculture sector in Malaysia

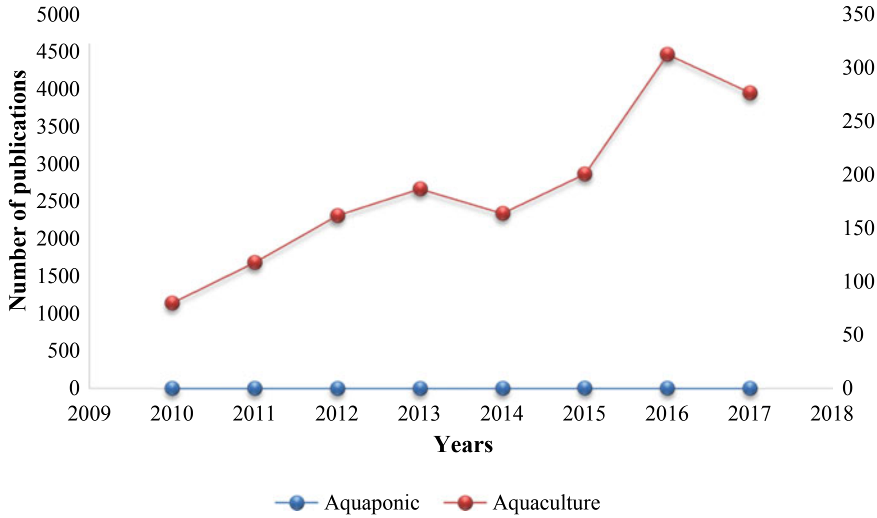

Fig. 8.1 Publication on aquaculture and aquaponics in Malaysia (*Source* http://www.sciencedirect.com, 2018, search terms 'aquaculture' and 'aquaponic' within these results)

is primarily associated with its economic gains as benefits of supplying domestic and foreign demands for the produce. According to Merino et al. (2012), as global fisheries yield is constrained by ecosystems' productivity and management effectiveness, per capita fish consumption can only be maintained or increased if aquaculture makes an increasing contribution to the volume and stability of global fish supplies. Therefore, due to the importance of this industry for the economic growth of the nation, it is envisioned to continue to grow in the expected future.

The main function of aquaculture industries is to improve the quality and quantity of wastewater and to fully utilize the existing land and water resources such as lake, pond, dams and river estuary (Fry et al. 2016). This leads to an expansion of the industry in Malaysia to maximize gains obtainable from the utilization of the nation's natural resources. Despite economic benefits that can be accumulated in the expansion of these industries, it should not consider the negative environmental impacts that follow lightly. Propagation of this sector must not compromise the state of the environment of this country to the extent of having unwanted irreversible impacts of the developments affecting the present livelihood and future generations. Therefore, the management of aquaculture should be taken seriously to maintain the biodiversity, especially for aquatic life. The common fish species cultured are red tilapia hybrid (*Oreochromis* sp.), catfish (*Clarias sp*.) and climbing perch (*Anabas testudineus*) (FAO 2008). However, it is noticeable that the development of aquaculture and aquaponics worldwide is a rollback as shown in Fig. 8.1.

The decline is due to several factors including lack in genetic management and poor hatchery procedures—particularly but not only in developing countries. These lacking have significantly degraded the performance of many farmed species through inbreeding, genetic drift and uncontrolled hybridization (Bostock et al. 2010). However, the growth of aquaculture for land-based and near-shore systems has peaked due to political, environmental, economic and resource constraints. According to Fig. 8.1, publications on the aquaculture in Malaysia were the highest in 2016 where 313 papers about aquaculture were published. However, research about aquaponics in Malaysia is still below the unsatisfactory level compared to the neighbouring countries. Among the number of publications on aquaponics in 2015, only five papers were published from Malaysia.

The development of aquaculture is currently ongoing, driven by new ideas and innovations. Integrated aquaculture is gaining attention as an innovated system to add value to water, recycle nutrients and wastes in the system to produce more crops. Integration of crops is also regarded as an environmentally friendly practice which intensifies the use of land (Ansar 2018). Integration of aquaculture and hydroponic is known as aquaponics. It combines the rearing of aquatic organisms (mainly fish) and plant production in a recirculating water system (Popp et al. 2018). The concept of aquaponics is to reuse the nutrient-enriched water from the fish rearing tank for the growth of plants in hydroponic system. In addition, aquaponics is a productive method of producing fish and vegetables in a green, sustainable and energy-efficient system. Aquaponics presents opportunities to increase economical operations because spaces, nutrients and water are optimized. This contributes to lower infrastructural costs, production of inexpensive food and consequently poverty reduction.

According to Goddek (2017), there are several designs available for aquaponic system. The designs are based on hydroponic systems, except that the water source for the aquaponic system comes from the fish tank and is eventually returned to its source of origin. There are three types of aquaponic systems, namely media filled systems, nutrient film technique (NFT) and floating raft system. In media filled system, the hydroponic component is distinguished first if it employs a media or not. According to Estim et al. (2018), the system removed 59% PO_4-N and 50% NH_3-N. Boxman et al. (2018) reported that *Sesuvium portulacastrum* (flower plant) was able to remove 59% N from marine fish wastewater.

The second system is the nutrient film technique (NFT). This system consists of the plant roots being exposed to a thin layer of nutrient water that runs through most often a PVC pipe. Based on previous studies by Buzby and Lin (2014) and Endut et al. (2010), these types of aquaponics needs a large area to build this system. In Buzby and Lin (2014), the system was able to remove 81% of NH_3-N by using lettuce and 89% by nasturtium. Meanwhile, Endut et al. (2010) claimed that the system removed total ammonia nitrogen (TAN) and total nitrogen (TP) by 86.22% and 49.74%, respectively. The shallow flow of water only reaches the bottom of the thick layer of roots that develops in the trough while the top of the root mass is exposed to the air, thereby receiving an adequate oxygen supply. Channel slope, length and flow rate must all be calculated to make sure the plants receive sufficient water, oxygen and nutrients. If properly constructed, NFT can sustain very high plant densities. In aquaponic NFT systems, the biofilter becomes crucial as there is no large surface area whereby bacteria communities can develop.

The floating raft system is another type which has great potential for commercial use in aquaponics (Wongkiew et al. 2017; Wang et al. 2016; Lam et al. 2015). The system plants are grown on floating styrofoam rafts. The rafts have small holes cut in them where plants are placed into net pots. The roots hang free in the water where nutrient uptake occurs. Wongkiew et al. (2017) claimed that pak choi and lettuce removed $NO_3$by 84% and 67%, respectively. Wang et al. stated that water spinach removed 80% of TAN by using this system. Marques et al. (2017) used flower plant, *Halimione portulacoides*, in their system and 67% of N removal was attained in the treatment of fish wastewater.

In summary, Estim et al. (2018), Wongkiew et al. (2017), Wang et al. (2016) and Lam et al. (2015) used floating raft system in aquaponic biofilter system. This is because the system is easy to set up, managed and monitored during the experiment. In addition, the types of plants also play an important role in nutrient removal. This is evident where flower plant, *Nasturtium* removed 89% of NH_3-N. While vegetable plant, water spinach removed 86% of NH_3-N. This is because the flower plant was able to stand higher temperatures up to 32 °C (Catley and Brooking 1996). Manarangi et al. (1988) found that higher temperature increased the number of flowers and their production rate. The efficiency of different systems used in previous studies including the plants involved is summarised in Table 8.1.

Table 8.1 Types of aquaponic systems and their efficiency reported in the literature from study conducted in Malaysia, Taiwan and United States

Type of aquaponics	Type of plants	Results	References
Media filled system, floating raft system, nutrient film technique	Green beans and Chinese cabbage	59.42% of PO_4-N removal and 50% of NH_3-N removal	Estim et al. (2018)
Media filled system	*Sesuvium portulacastrum*	59% of N removal	Boxman et al. (2018)
Floating raft system	Pak choi and lettuce	84% of NO_3 removal for pak choi and 67% of NO_3 removal for lettuce	Wongkiew et al. (2017)
Floating raft system	*Halimione portulacoides*	67% of N removal	Marques et al. (2017)
Floating raft system	Water spinach	60% of TP removal and 70% of TN	Lam et al. (2015)
Floating raft system	Water spinach	80% of TAN removal	Wang et al. (2015)
Nutrient film technique	Lettuce and *Nasturtium*	81% of TAN removal for lettuce and 89% of TAN removal for nasturtium	Buzby and Lin (2014)
Nutrient film technique	Water spinach	86.22% of TAN removal and 49.74% of TP removal	Endut et al. (2009)

8.4 Conventional Nitrogen Removal Technologies

The latest nitrogen removal technologies can be categorized into physico-chemical or biological. There are six types of physiochemical technologies and seven types of biological technologies. The advantages and limitations of each technology are described in Table 8.2. Gupta et al. (2015) stated that ion exchange, adsorption and biological technology are among the most frequently studied technologies for wastewater treatment of ammonium ions. However, these technologies are costly in terms of production, operation and maintenance because they require chemical reagents or complementary facilities (Mishra and Maiti 2017). Provolo et al. (2017) studied on ammonia air stripping method in manure wastewater. The result from the previous study showed 69% of ammonia removal but the method is expensive because it requires high technology equipment. Huang et al. (2014) studied on ammonia removal from landfill leachate by using struvite precipitation. Ammonia removal was 82% and the method required various chemical reagents. Rahmani and Mahvi (2006) performed a study in domestic wastewater by using ion exchange process. The study reported that up to 87.7% of ammonium removal was achieved but this method required a suitable cationic concentration to increase the ammonium removal capability. Haseena et al. (2016) demonstrated the adsorption method in the removal

Table 8.2 The advantages and disadvantages of nitrogen removal technologies

Nitrogen removal technologies	Advantages	Disadvantages	Result	References
Physiochemical means				
Ammonia air stripping	Treatment of wastewaters with high ammonium concentration Simple operation and effective	High cost for ammonia recovery Icing of the lower packing strata in freezing conditions	69% of ammonia removal	Provolo et al. (2017)
Ammonia precipitation as struvite	Able to treat concentrated wastewater (digested sludge liquor, landfill leachate)	The cost of adding magnesium salts is the major economic constraint Struvite precipitation can be influenced by impurities such as calcium	82% for ammonia removal	Huang et al. (2014)
Ion exchange process	Highly efficient and simple process Good control of effluent quality No sensitivity in fluctuation of NH_4^+ influent concentration	High cationic concentration (potassium, calcium, sodium, magnesium) of the solution will reduce ammonium removal capability	87.7% of ammonium removal	Rahmani and Mahvi (2006)
Adsorption	Effective and economic method Flexible in design and operation	Only applicable to wastewater with low AN content Regeneration process is not available for all types of adsorbents	82.11% of ammonia nitrogen removal	Haseena et al. (2016)

(continued)

Table 8.2 (continued)

Nitrogen removal technologies	Advantages	Disadvantages	Result	References
Chemical oxidation of ammonia	Simple design of mechanical components for breakpoint chlorination system Promising and innovative technologies	This process is practical only as an effluent polishing technique, not for the removal of high levels of nitrogen Advanced oxidation process (photocatalytic oxidation and electrochemical oxidation), although very promising, still confined to the level of applied research	85.2% of ammonia nitrogen removal	Chen et al. (2018)
Chemical reduction of nitrate	Low cost and lack of sensitivity to other impurities Absence of limitation by nitrate concentration level	Still confined to the level of applied research	88% of nitrate removal	Hamid et al. (2018)
Biological means				
Nitrification and denitrification	Environmentally friendly and cost-effective	May become complicated when applied to industrial wastewater due to the varied characteristics Slow	Approximately 100% of total nitrate removal	Rysgaard et al. (1993)
Simultaneous nitrification and denitrification (SND)	Low biomass yield during anaerobic growth Effective in maintaining a neutral pH level in the reactor	Requirement of advanced bioreactor with adjustable aerobic, buffer and anoxic zone with liquid circulation	75.09% of total nitrogen removal	Li et al. 2016

(continued)

Table 8.2 (continued)

Nitrogen removal technologies	Advantages	Disadvantages	Result	References
Autotrophic denitrification	Less sludge production Low biomass build-up	Wastewaters have a very low concentration of biodegradable organic material requires addition of an electron donor substrate	98.1 ± 0.4% of nitrogen removal	Sun et al. (2018)
Anaerobic ammonium oxidation (Anammox) process	Lower operating cost compared to nitrification and denitrification process Ideal for wastewater with C:N ratio < 1	Long start-up times are required to obtain a sufficient biomass concentration due to low biomass yield	82.5% of total nitrogen removal	Ye et al. (2018)
Partial nitrification and anammox process	Better system control of the separate processes compared to CANON process	Not economic due to the need for two reactors	89% of total nitrogen removal	Ding et al. (2018)
Completely autotrophic nitrogen removal over nitrite (CANON) process	Lower N_2O emissions Reduce risk of anammox bacteria inhibition	Require long retention times to prevent the wash out of the suspended nitrifiers from the reactor	93.4% of ammonium nitrogen removal and 81% of total nitrogen removal	Yue et al. (2018)
Simultaneous nitrification, anammox and denitrification (SNAD)	Able to achieve nitrogen removal efficiency higher than 89% under autotrophic condition	Operational limits that guarantee the stability of the process are not well defined to date	72 ± 2% of total nitrogen removal	Wang et al. (2018)

of ammonia nitrogen (AN) in lakes affected by agriculture activities. They reported 82.11% removal; however, this method is only applicable to water containing low AN. Chen et al. on the other hand, claimed that chemical oxidation of ammonia method removed 85% of ammonia nitrogen but the problem is still confined to the level of the applied research.

Rysgaard et al. (1993) examined the nitrification and denitrification method in lake and estuarine and obtained approximately 100% of nitrate removal. However, this method may become complicated when applied to industrial wastewater due to its various characteristics. Li et al. (2016) applied simultaneous nitrification and denitrification (SND) to remove total nitrogen up to 75.09%. This process is complicated because it requires advanced bioreactor with adjustable aerobic, buffer and anoxic zone with liquid circulation. Meanwhile, Sun et al. (2018) demonstrated autotrophic denitrification method and the result was $98.1 \pm 0.4\%$ of nitrogen removal.

The current review discusses the performances of conventional methods in terms of nitrogen removal including ammonia, nitrite and nitrate. Among the conventional methods, nitrification and denitrification have high potential for nutrients removal. They are environmentally friendly and cost-effective but these methods are time-consuming. The advantages and disadvantages of nitrogen removal technologies are listed in Table 8.2.

8.4.1 Nutrient Removal

Nutrient removal refers to the removal of nitrogen or phosphorus in the treatment of the wastewater. Excessive nitrogen or phosphorus can cause nuisance growth of algae or weeds in the receiving water. Discharges containing nitrogen and phosphorus may accelerate the eutrophication of lakes and reservoirs and may stimulate the growth of algae and rooted aquatic plants in shallow stream (Apandi et al. 2019). In addition, the presence of algae and aquatic plants may interfere with the beneficial uses of the water resources especially when they are used for water supplies (Abdel-Raouf et al. 2012). Nitrogen in wastewater is mostly in the form of ammonia and organic nitrogen. These nitrogen forms can be converted into nitrate nitrogen by bacteria, if the plant is designed to provide enough oxygen with a long enough "sludge age" to develop these slow-growing plant nutrient. The nitrate nitrogen is less toxic than ammonia. If more complete removal of nitrogen is required, a biological process can be set up. This biological setup can reduce the nitrate to nitrogen gas (and some nitrous oxide). The nitrogen convention is shown in Fig. 8.2. Nitrification is the biological oxidation of NH_4^+ to NO_3^- through a two-step autotrophic process by the bacteria *phosph* and *Nitrobacter* (Zhang et al. 2018).

A two-step reaction is usually very rapid; hence, it is rare to find nitrite levels higher than 1.0 mg/L in water (Luo et al. 2015). The nitrate formed by nitrification is used by plants in the nitrogen cycle as a nitrogen source (synthesis) or reduced to N_2 gas through denitrification process. NO_3^- can be reduced under anoxic conditions

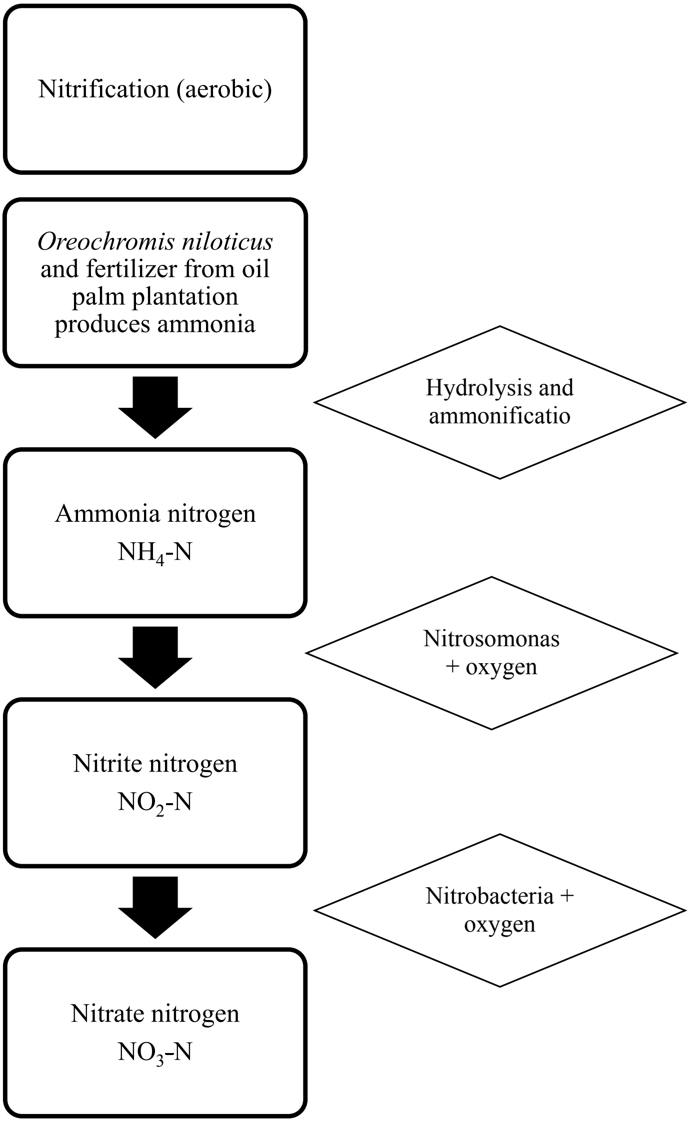

Fig. 8.2 Nitrification process

to N_2 gas through heterotrophic biological denitrification as shown in the following unbalanced equation (US EPA 1993):

$$NO_3^- + \text{Organic matter} \rightarrow N_2 + CO_2 + OH^- + H_2O \tag{8.1}$$

The above equation is identical to the equation for biological oxidation of organic matter with the exception that NO_3^- is used as an electron acceptor instead of O_2:

$$O_2 + \text{ Organic matter} \rightarrow CO_2 + OH^- + H_2O \tag{8.2}$$

A large variety of heterotrophic bacterias can use nitrate in lieu of oxygen for the degradation of organic matter under anoxic conditions. If O_2 is present, however, the bacteria will preferentially select it instead of NO_3^- (US EPA 1993). Thus, it is very important that anoxic conditions exist so that NO_3^- will be used as electron acceptor. Autotrophic denitrification is also possible with either elemental sulphur or hydrogen gas used as electron donor by autotrophic bacteria as shown in the following unbalanced equation (Li et al. 2016):

$$NO_3^- + CO_2 + \text{ Inorganic Electron Donor } \rightarrow N_2 + \text{ Oxidized Electron Donor} \tag{8.3}$$

8.5 Selection of Tilapia (*Oreochromis Niloticus*) for Aquaponic Biofilter System

The type of fish used in an aquaponic system is dependent on the surrounding climate. The temperature shall be able to maintain: the types of fish which local fisheries department has specified as legal (there are sometimes restrictions on the cultivation of fish that are not native to the region), the type of fish desired for consumption by consumers and the type of fish feeds available to the grower (Yavuzcan et al. 2017). The hybrid striped bass is one species that has been reported as cannot perform well in aquaponic systems because it cannot tolerate high potassium levels which are common supplements used for plant growth (Dediu et al. 2012).

Tilapia is the second most cultured freshwater finfish species after carps. It has the potential to be the most important farmed fish in the twenty-first century (Mahieu 2015). The worldwide production of tilapia fish in 2015 was estimated at 6.4 million metric tonnes (MMT), (FAO 2017a). Tilapia is considered to be relatively resistant to a few numbers of diseases encountered in other cultured fishes (Del-Pozo et al. 2017). One of the specialties of tilapia is hypoxia tolerance (ability to live in critically low level of oxygen). The hypoxia tolerance of tilapia is higher than other aquatic organisms, and tilapia can tolerate a hypoxia environment with dissolved oxygen below than 0.5 mg/L for a short period (Lim et al. 2006). This makes it a suitable model fish to be used in studying ammonia removal in an aquatic habitat. Other than that, tilapia is selected by studies due to the availability of its genome database (Chen et al. 2017).

Table 8.3 Culture condition of tilapia (*Oreochromis niloticus*) in previous studies

Type of fish	Condition of culture	Survival rate	References
Oreochromis niloticus	29.5–30.2 °C pH: 6.83–7.00 DO: 6.10–6.37 mg/L	NR	Effendi et al. (2018)
Oreochromis niloticus	25.2–25.9 °C pH: 6.9–7.2 DO: 6.5–6.8 mg/L	93.3 ± 4.4%	Estim et al. (2018)
Oreochromis niloticus	28.9–30.5 °C pH: 8.7–8.8 DO: 5.87–6.44 mg/L	100%	Silva et al. (2018)
Oreochromis niloticus	26.0–26.2 °C pH: 7.1–7.2 DO: 5.4–5.6 mg/L	100%	Hu et al. (2015)

NR = Not Reported

Tilapia is a tropical fish originating from the Near East and Africa that can adapt well to aquaponic system and is exceptionally resilient against fluctuations in dissolved oxygen levels, temperature, pH and dissolved solids. The temperature range that tilapia enjoy also correlates to ideal temperatures for the growth of aquaponic plants. Tilapia is the fastest growing species used in aquaponic systems (Hu et al. 2015). The white-fleshed meat of tilapia is popular due to its desirable culinary properties of taste and texture. Therefore, tilapia is the 6th most fish culture species in the world (Reantaso 2017). A member of the cichlid family, tilapia is the most widely cultured fish in tropical and subtropical areas of the world and has also been introduced to Japan, India and throughout Asia, Russia, Europe and the America (De Silva 2012) (Table 8.3).

8.6 Applications of Plant for Ammonia Removal

The ability of plants to absorb pollutants from lake water is well-known as a low-cost and eco-friendly remediation technique. Many studies have demonstrated that plants contribute to water treatment through direct and indirect mechanisms and thus be an essential part of a system. Excess amount of TN in wastewater can be incorporated to plant biomass and increase the plant growth (Holm and Heinsoo 2013). Various plant species have been investigated for N removal. For that purpose, plants with high transpiration capacity and commercial benefits are top candidates (Holm and Heinsoo 2013; Konnerup et al. 2009). For example, Heliconia ornamental plants (*Heliconia psittacorum*) have been reported to remove 13% of N when they were applied for the treatment of domestic wastewater containing 27 mgL^{-1} N (Konnerup et al. 2009). Peña-Salamanca et al. (2013) stated that *H. psittacorum* removed 33% of ammonia nitrogen from high-strength wastewater in tropical areas.

Table 8.4 Previous studies on *Heliconia psittacorum* to remove pollutants

Type of plants	Results	Researchers
Heliconia psittacorum	Removal of 72% ammonia nitrogen	Madera-Parra et al. (2015)
Heliconia psittacorum	Removal of 13% of N	Konnerup et al. (2009)
Heliconia psittacorum	Removal of 33% of ammonia nitrogen	Peña-Salamanca et al. (2013)

Heliconia sp. stands out among tropical flowers due to its high consumer acceptance, due to its shape, intense colouring and exoticism of the inflorescence, including its high post-harvest durability. According to Madera-Parra et al. (2015), *Heliconia psittacorum* has been reported for the removal of 72% ammonia nitrogen when applied in phytoremediation of landfill leachate. Therefore, this makes Heliconia species among flower plants with high potential of nutrient removal (phytoremediation). The most important functions of the plants are related to their physical effects in the lake water. The roots provide a huge surface area for the attached microbial growth, and in temperate regions, the plant litter provides an insulation layer against frost during winter. Plants can also facilitate aerobic degradation by releasing oxygen to the rhizosphere, but oxygen release rates are difficult to be quantified and the overall effect on pollutant removal is probably varying (El-Khateeb and El-Bahrawy 2013). Regarding the uptake of nitrogen (N) and phosphorus (P), many studies in temperate climate showed that the amount which can be removed by harvesting is generally insignificant (Geng et al. 2017). However, in tropical climates where the plants grow faster and throughout the year, the uptake of nutrients can probably contribute to significantly higher removals of nutrients as has been reported in several studies. However, if the plants are not harvested, the incorporated nutrients will be released again during decomposition of the biomass (Table 8.4).

8.6.1 Temperature Effect on Plant Growth

Among the naturally occurring environmental factors, temperature is considered to play a predominant role in controlling proper growth and flowering. Most *Heliconia* species could not tolerate cold weather and will suffer injury when temperature falls below 13 °C. Criley and Broschat (1992) and Manarangi et al. (1988) found that higher temperatures increased the number of flowers and their production rate. The general climatic conditions are required for healthy growth are warm and humid.

8.6.2 Photoperiod Effect on Plant Growth

Photoperiodism is one of the most significant and complex aspects of the interaction between plants and their environment. It is defined as plant responses to day length, enabling living organisms to adapt to seasonal changes. In *Heliconia psittacorum* case, it has been reported that the main factors influencing flowering are irradiance and temperature. However, photoperiod also appears to have a slight influence (Xu et al. 2016). The most widely used photoperiods are 12 h of light/12 h of darkness or 16 h light/8 h of darkness (De Silva et al. 2012).

In most of the studies carried out using different species of *Heliconia*, the photoperiod used was 16 h. In *Heliconia stricta*, a seasonal pattern of flowering has been recorded, yields are greater for plants grown at 8 h photoperiods than under natural day length. No flowering occurred in long days. For *Heliconia aurantiaca*, it has been observed that under glasshouse conditions in Denmark, flowering only occurs during November to February. This pattern suggests that flowering might be influenced by photoperiod. Since *Heliconia latispatha* is an attractive plant that shows potential as a cut flower, particularly if flowering could be extended to a greater part of the year.

8.6.3 Concept of Phytoremediation Technology

Phytoremediation is known as a naturally occurring process and being documented by humans for more than 300 years ago (Bindu 2004). Since then, some plants' capabilities to survive in polluted areas and to ease pollutants removal from the environment had been discovered by humans. The number of plants depends on the size of the space available and the desired plant density. However, greater plant densities will achieve canopy closure more rapidly, therefore, water uptake will also reach a maximum rate more quickly. Lu et al. (2008) stated that there are three principles for operating an aquatic phytoremediation system. Firstly, implementation and identification of efficient aquatic plant systems. Secondly, uptake of dissolved nutrients including N, P and metals by growing plants. Lastly, harvest and beneficial use of the plant biomass produced from the remediation system. In order to ensure optimum plant density, the regular harvest grown-up biomass from water bodies is necessary. Otherwise, the dead plant tissues will decompose and then release the stored nutrients back to the environment.

There are five types of phytoremediation mechanisms for pollutant removal, namely, phytovolatilization, phytodegradation, phytoextraction, rhizofiltration and phytostabilization. Phytovolatilization is the extraction, and subsequent compounds are released in gaseous form from the foliage into atmosphere. Phytodegradation is the conversion of organic pollutants into non-toxic forms by plants and associated microorganisms which occurs at rhizosphere or plant internals. Meanwhile, phytoextraction is the natural ability of plant to take up substances (e.g. organic compounds) from the environment and followed by sequestration of those substances inside plant cells. Rhizofiltration is the sorption of contaminants onto root surface or other plants parts, or the precipitation in the root zone. Lastly, phytostabilization is the immobilization of compounds in surrounding environment through the binding of the contaminants and chemicals released by the plant. Mayo and Hanai (2017) conducted experiment on nitrogen removal and 73.8% was succeeded to be removed from waste stabilization pond. Meanwhile, Chen et al. (2017) claimed to remove 57.7% of nitrogen from drainage water. Ojoawo et al. (2015) studied on phytoremediation of domestic wastewater and removed 51.9% of nitrate. Fang et al. (2007) stated that phytoremediation removed 76.2% of total nitrogen from eutrophicated water. Depending on the type of pollutants to be remediated by plants, the mechanisms used are also different, described in Table 8.5.

Phytoremediation is recommended since natural plants are used compared to conventional methods which need costly chemical reagents to remove nitrogen present in lake water. Table 8.6 presents the retention time of phytoremediation by several researchers to remove pollutants. In the phytoremediation process to remove pollutants, there are two main limitations that require consideration: slowness and non-applicability to wastewater with extremely high pollutant content. According to Mayo and Hanoi (2017) phytoremediation process commonly consumes longer retention time ranging from 10 days to 3 years for ammonia removal. Ojoawo et al. (2015) also studied on phytoremediation by using *Canna* sp. was and able to remove 52% of nitrate in 28 days. Therefore, phytoremediation has been recorded as the fastest method to remove nutrient in 10 days by using natural treatment and more practicable method.

8.6.4 Previous Studies on Biofilter and Its Ability to Remove Pollutants

Previous research of treatments specifically on ammonia removal by using the same method but different keywords such as phytoremediation and constructed wetland are illustrated in Table 8.7. In Sim et al. (2008), *Phragmites karka* and *Lepironia articulate* were applied in Putrajaya City, Malaysia to treat agricultural wastewater. From the study, the plants used can remove 82.11% and 84.32% for total-N (TN) and total-P (TP), respectively. Konnerup et al. (2009) demonstrated the ability of *Heliconia psittacorum* and *Spathocircinata* for nitrogen (N) removal applied in domestic wastewater and obtained 41% of N removal. Varun and Kalpana (2015) had conducted a study by using water hyacinth on domestic wastewater. The study focused on the mechanism of phytoremediation and the result showed that the plant removes up to 67% of ammonia nitrogen (AN). Besides that, Yavari et al. (2017) reported that using the same mechanism of phytoremediation to treat urea manufacturing wastewater by using *Tectona grandis*. The plant was able to remove up to 47% and 48% for TN and TP, respectively. Furthermore, *Spartina alterniflora* and *Juncus roemerianus* also have been studied for their performance in the removal of TN and TP. These

Table 8.5 Different phytoremediation mechanisms used to remediate varying pollutants

Type of contaminants	Mechanisms	Results	References
Organic compounds	Phytoextraction: Direct uptake and accumulation of contaminants and metabolism in plant tissues Phytovolatilization: transpiration of volatile organic compounds (VOC) through the leaves Rhizosphere bioremediation: release of exudates that stimulate microbial activity and biochemical transformations in the soil Phytotransformation: Enhancement of mineralization into relatively non-toxic constituents such as carbon dioxide, nitrate, chlorine and ammonia at the root-soil interface	71% of volatile organic compound (Formaldehyde)	Teiri et al. (2018)
Nitrogen	Biological: ammonification, nitrification, denitrification, plant uptake, biomass assimilation, dissimilatory nitrate reduction Physico-chemical: ammonia volatilization, adsorption	73.8% of nitrogen removal from waste stabilization pond	Mayo and Hanai (2017)
		51.9% of nitrate removal from domestic wastewater	Ojoawo et al. (2015)
		76.2% of total nitrogen from eutrophicated water	Fang et al. (2007)
		57.7% of nitrogen removal from drainage water	Chen et al. (2017)

plants were applied on marine aquaculture solid waste and the result showed for TN removal was very low approximately 4% but was the highest at approximately 52% for TP removal. Effendi et al. (2018) used *Vetiveria zizanioides* on tilapia cultivation wastewater. The result showed that the plant was able to remove ammonia nitrogen at 30.17%.

Table 8.6 The retention time for pollutant removal by phytoremediation

Methods	Results	Researchers
Phytoremediation (*Eichhornia crassipes*)	10 days to 3 years of retention time to remove ammonia	Mayo and Hanai (2017)
Phytoremediation (*Cladophora* sp.)	10 days of retention time to remove arsenic from 6 to <0.1 mg/L	Jasrotia et al. (2017)
Phytoremediation (*Canna* sp.)	28 days to remove 52% of nitrate	Ojoawo et al. (2015)

Table 8.7 Previous studies on constructed wetland for nitrogen removal

Type of wastewater	Type of plants	Characteristics	Result	References
Tilapia cultivation wastewater	*Vetiveria zizanioides*	• Centre for environmental research, Bogor, Indonesia • Freshwater • Tropical climate	30.17% of AN Removal	Effendi et al. (2018)
Urea manufacturing wastewater	*Tectona grandis* (phytoremediation)	• Located in Universiti Teknologi Petronas • Freshwater	47% of N removal 48% of P removal	Yavari et al. (2017)
Domestic wastewater	*Phragmites australis* (constructed wetland)	• Located in Sarnadas Rodao, Portugal. • Freshwater • Temperate climate	10.4% of total-N removal 27.5% of total-P removal	Mesquita et al. (2017)
Marine aquaculture solid waste	*Spartina alterniflora Juncus roemerianus*	• Located in Atlantic and Gulf of Mexico coasts • Marine • Temperature: 18.2–24.2 °C	4% of total-N removal 52% of total- P removal	Joesting et al. (2016)
Domestic wastewater	Water hyacinth (phytoremediation)	• Located in Research Institute University, Chennai	67% of AN removal	Varun and Kalpana (2015)
Domestic wastewater	*Heliconia psittacorum Spathocircinata* (constructed wetland)	• Located at the Asian Institute of Technology (AIT) in Thailand • Tropical temperature	41% of N removal	Konnerup et al. (2009)
Agricultural wastewater, domestic wastewater, urban surface runoff	*Phragmites karka Lepironia articulate* (constructed wetland)	• Located in Putrajaya City, Malaysia. • Freshwater	82.11% of total-N removal 84.32% of total-P removal	Sim et al. (2008)

8.7 Conclusion

This review article has been pursued to give encyclopaedic idea about the biofilter aquaponic system in the entire country, so readers can get a perception and impression of the important role of plants for removing pollutants in wastewater. Few studies have been focused on the plant species in biofilter aquaponic system. Therefore, this aspect needs to be investigated and further studied to facilitate the best selection of treatment processes and understand their impact on the environment. Furthermore, fresh market wastewater with high content of nutrient and pathogenic substances need to be treated properly before being discharged to water bodies system and should be used as mandatory practice for cleanliness environment. As a conclusion, the types of plants function as a nutrient remover have been proved by several studies. The studies confirm that the selected plants are suitable for wetland treatment in a tropical climate. The constructed wetland treatment system is a cheaper alternative for wastewater treatment using local resources and is an energy-efficient technology. Constructed wetlands offer multiple values such as the creation of habitat, water quality improvement, flood control, and production of food and fibres (also termed as constructed aquaculture wetlands). However, long term monitoring and maintenance are crucial to ensure the performance of the wetlands.

Acknowledgements The authors would like to express their appreciation to the Micropollutant Research Centre (MPRC), Universiti Tun Hussein Onn Malaysia (UTHM) for the research facilities. The authors also would like to thank the Research Management Centre (RMC) Universiti Tun Hussein Onn Malaysia (UTHM) for the financial support under Grant GPPS (U957).

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Chapter 9
Struvite Crystallization: An Effective Technology for Nitrogen Recovery in Landfill Leachate

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Abstract The presence of different sorts of nutrients in wastewater raises the concern on adopting effective treatment techniques, to prevent potential adverse impacts on the environment's components, as well as to recycle nutrients for useful applications. Nitrogen is a popular nutrient that often existed abundantly in landfill leachate. Struvite ($MgNH_4PO_4 \cdot 6H_2O$, MAP) crystallization is a common technology that is widely applied for nitrogen recovery in landfill leachate, due to its high efficiency, simplicity and environmental sustainability. This chapter emphasizes the role of MAP technology in the treatment of $NH_4{-}N$ in landfill leachate. Moreover, the physico-chemical fundamentals of MAP crystallization are explained thoroughly. In addition, the recent enhancements and related developments are discussed in detail. Besides, the contribution of MAP technology in treatment of other wastewater parameters is also discussed. In order to point out the cost-effectiveness of this technology, the options of cost saving and reduction are analyzed through this chapter.

Keywords Struvite · Nitrogen · Phosphorus · Landfill leachate · Crystallization · Fertilizer

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A. A. S. Al-Gheethi et al. (eds.), *Prospects of Fresh Market Wastes Management in Developing Countries*, Water Science and Technology Library 92,
https://doi.org/10.1007/978-3-030-42641-5_9

9.1 Introduction

Nitrogen is considered as a key nutrient that is necessary for all forms of life. It is a basic element for many vital industries such as foods and fertilizers. On the other hand, N is one of the most harmful pollutants for humans and aquatic environment. Disposal of high N loads to natural water bodies promotes eutrophication, which results in oxygen depletion and toxicity to aquatic life. Ammonium nitrogen represents more than 80% of total nitrogen in wastewater (Gunay et al. 2008a; Zhang et al. 2012), which means that recovery of $NH_4{-}N$ should be highly considered.

Landfilling is the most widely applied method for solid wastes disposal. Globally, about 60% of generated solid wastes are disposed in landfills. Corresponding to pollutants migration into the environment, landfills produce two main hazardous wastes: the biogas (formed by the fermentation of organic matter), a source of air pollution; and the leachate (generated by the contact of percolated water with the decomposing wastes), a source of groundwater and soil contamination. Landfill leachate is one of the hazardous wastewaters that contains high concentrations of $NH_4{-}N$ (Li et al. 1999). In their study, Alslaibi et al. (2011) revealed that $NH_4{-}N$ concentration (>8 mg/L) was higher than natural concentration (0.2 mg/L) in groundwater, near landfill sites in Gaza Strip, Palestine. In Malaysia as well, it has been found that LL pollutes some rivers and increases the concentration of $NH_4{-}N$ to above the Interim National Water Quality Standard (INWQS) Class IIA/IIB river for Malaysia (>0.3 mg/L) (Yusof et al. 2009). These studies showed obviously the high risk of disposing LL without a proper treatment of $NH_4{-}N$. For these reasons, recovery of $NH_4{-}N$ from LL is crucial for environmental protection.

9.2 Characteristics of Landfill Leachate

Fundamentally, physical and chemical characteristics of LL are strongly influenced by the composition of wastes, climate conditions and landfill age. Age of landfill is the driving factor, and accordingly, LL can be classified into three main types: young, medium-aged and old (mature). Besides, LL is usually characterized by the basic parameters, which are pH, COD, BOD, $NH_4{-}N$, total Kjeldahl nitrogen (TKN), heavy metals and SS.

Table 9.1 presents the main characteristics of LL in some literatures. It can be noticed that the age of landfill, and hence the degree of wastes stabilization, has a significant effect on LL characteristics. It is also important to mention that $NH_4{-}N$ constitutes nearly 90% of total nitrogen in old LL (Gunay et al. 2008a; Siciliano et al. 2013), which indicates that the organic content is almost completely stabilized. The ratio of BOD_5/COD is much lower in stabilized LL due to the release of recalcitrant organics from the solid wastes. Therefore, old LL can be characterized by the low ratio of BOD_5/COD and the high concentration of $NH_4{-}N$, while young and medium-aged leachates often have higher BOD_5/COD ratios.

Table 9.1 Characteristics of landfill leachate

Age*	COD (mg/L)	BOD_5 (mg/L)	B/C‡ (mg/L)	TKN# (mg/L)	NH_4-N (mg/L)	pH	References
Y	18,565	10,550	0.57	2,500	2,220	8.0	Kabdaşli et al. (2008)
Y	20,400	15,000	0.74	2,950	2,700	7.3	Gunay et al. (2008b)
Y	42,500	23,000	0.55	2,370	2,020	6.3	Öztürk et al. (2003)
MA	5,500	1,250	0.38	2,218	2,083	9.2	Akkaya et al. (2010)
MA	9,257	3,192	0.35	3,298	2,388	8.1	Barnes et al. (2007)
MA	5,050	1,270	0.25	1,670	1,330	8.4	Frascari et al. (2004)
O	4,537	521	0.12	N.A.**	3,126	8.0	Li et al. (2012)
O	1,365	276	0.20	N.A.	1,320	7.5	Kamaruddin et al. (2013)
O	9,700	560	0.06	N.A.	2,620	8.16	Siciliano et al. (2016)

*Y: Young, MA: Medium-aged, O: Old. **N.A.: Not available
‡B/C: BOD_5/COD. #TKN: Total Kjeldahl Nitrogen

9.3 Struvite Precipitation in Wastewater

Chemical precipitation approach has been successfully applied for NH_4-N recovery in the form of struvite, especially from highly polluted wastewaters such as LL (Di Iaconi et al. 2010), because of the simplicity and high efficiency of this method (Li et al. 2012). In addition, MAP crystallization is a nitrogen-selective removal method that is not achievable in many other methods. Besides, a partial reduction of other pollutants in wastewater was achieved, including chemical oxygen demand (COD) (Ryu et al. 2008), Total Carbon (TC) and Total Organic Carbon (TOC) (Zhang et al. 2012), SS, colour and turbidity (Gunay et al. 2008a). To date, struvite precipitation has been applied to recover NH_4-N in different types of wastewater as shown in Table 9.2.

In wastewater treatment plants, struvite has always been a scaling problem. It initially appeared as a “crust of crystalline material” in a pipe carrying supernatant liquors of a digestion system, which was identified as MAP (Rawn et al. 1937). Struvite was later confirmed by Borgerding (1972), who detected it on the walls of an anaerobic digester at a treatment plant in Los Angeles in 1963. So, struvite was initially considered more of a problem to eliminate (using acidic treatment) before its economic benefits were explored.

The operational cost of ammonia treatment is a significant factor that should be considered for any applied method. It is worthy to mention that the current applied techniques for ammonia treatment in LL cost around 4.5–12 USD/m^3 (Wu et al. 2016), which has to be reduced through innovative approaches. One of these approaches is an integrated system, developed by Wu et al. (2016), which removes N from LL using biological and electrochemical techniques. The authors could reduce the operational cost to 0.8 USD/m^3, regarding struvite technology. Huang et al. (2012) mentioned that, if the conventional reagents were applied, the operational cost would be more than 10 USD/m^3. However, based on the estimations of Huang et al. (2012), MAP technology is more competitive when low-cost Mg and P reagents are applied, or when struvite is recycled, at which the operational cost could be reduced to 0.94 USD/m^3. Furthermore, a combination of cost reduction methods could reduce the operational cost to more significant values (Table 9.2).

9.3.1 Struvite Characteristics

Struvite is an orthophosphate crystalline mineral with the chemical formula of $MgNH_4PO_4 \cdot 6H_2O$, which takes the orthorhombic structure (i.e. straight prisms with a rectangular base). The main characteristics of struvite are shown in Table 9.3. Struvite is a thermally unstable compound; its structure and morphology can be altered at a temperature of around 55 °C (Bhuiyan et al. (2008).

Struvite was later confirmed by Borgerding (1972), who detected it on the walls of an anaerobic digester at a treatment plant in Los Angeles in 1963. So, struvite was initially considered more of a problem to eliminate (using acidic treatment) before its economic benefits were explored. In practice, the presence of struvite mineral in precipitates can be inspected using different analytical techniques such as X-ray diffraction (XRD) (Capdevielle et al. 2013), X-ray fluorescence (XRF) (Liu et al. 2013a, b), Fourier transform Infrared (FT-IR) and thermogravimetric analysis (TGA) (Wang et al. 2010). The shape of the crystals is usually explored using scanning electron microscopy (SEM). Besides, quantitative analysis with high level of accuracy can be carried out by XRD (Lu et al. 2016; Yan and Shih 2016). However, other techniques such as inductively coupled plasma (ICP) and atomic absorption spectrophotometry (AAS) should be used to detect the exact content of struvite in the solid precipitates. Hao et al. (2008) developed an acidic dissolution method to analyze the elemental composition of struvite, which enables to evaluate its purity.

Struvite has been involved in other fields of study. For instance, it has often been studied in the medical field as it is one of the main components of the urinary stones (Suguna et al. 2012). Additionally, it has been applied in soil treatment field for heavy metals immobilization, which showed high efficiency in the immobilization of Cu, Pb and Cr (Wang et al. 2016).

Table 9.2 Recent studies on NH_4-N removal by struvite recovery for different types of wastewater

Wastewater type	Optimum		Mg source	P source	Reaction time*	NH_4-N		References
	pH	Mg:N:P				Initial concentration (mg/L)	Removal (%)	
Landfill leachate	9.5	1.15:1:1	$MgCl_2 \cdot 6H_2O$	$Na_2HPO_4 \cdot 2H_2O$	50 s	2,520 ± 100	86	Zhang et al. (2009a, b)
	10.0	1:1:1	$MgCl_2 \cdot 6H_2O$	NaH_2PO_4	30 min	610–640	88	Chen et al. (2013)
	9.0	1:1.2:1.2	$MgCl_2 \cdot 6H_2O$	H_3PO_4 (75%)	2 h	2,620	92	Siciliano et al. (2016)
	9.0	2:1:1	MgO	H_3PO_4	30 min	2,600	95	Di Iaconi et al. (2010)
	8.0**	1.4:1:0.8	MgO	NaH_2PO_4	1 h	3,126	85	Li et al. (2012)
	7.82	1:1:1.05	$MgCl_2 \cdot 6H_2O$	NaH_2PO_4	–	2,269	89	Jaafarzadeh et al. (2010)
	9.0	1.2:1:1.2	$MgCl_2 \cdot 6H_2O$	$Na_2HPO_4 \cdot 12H_2O$	13 min	1,500	96.6	Akkaya et al. (2010)
	10	1.3:1:1.3	Bittern (97% $MgCl_2$)	Bone-meal waste	1 min	2,600	90	Siciliano et al. (2013)
	8.5	1:1:1	Magnesit (98% $MgCO_3$)	H_3PO_4	15 min	2,600	98	Gunay et al. (2008b)
	8.5	1:1:1	$MgCl_2 \cdot 6H_2O$	$Na_2HPO_4 \cdot 12H_2O$	25 min	2,600	98	Gunay et al. (2008a)
Digested dairy manure	10.0	1:1.2:1	$MgCl_2 \cdot 6H_2O$	$Na_3PO_4 \cdot 12H_2O$	40 min	1,013–1,426	87	Zhang et al. (2012)

(continued)

Table 9.2 (continued)

Wastewater type	Optimum		Mg source	P source	Reaction time*	NH_4-N		References
	pH	Mg:N:P				Initial concentration (mg/L)	Removal (%)	
Semiconductor wastewater	9.2	1.2:1:1	$MgCl_2 \cdot 6H_2O$	K_2HPO_4	30 min	154	89	Ryu et al. (2008)
UASB pretreated Poultry manure	9.0	1:1:1	$MgCl_2 \cdot 6H_2O$	KH_2PO_4	15 min	1,318	85.4	Yetilmezsoy et al. (2009)
Saponification wastewater	9.0	1.05:1:1.05	$MgSO_4 \cdot 7H_2O$	$Na_2HPO_4 \cdot 12H_2O$	7 min	4,100	91.6	Huang et al. (2011a, b)
Human urine	8.5	1.2:1:1	$MgCl_2 \cdot 6H_2O$	$Na_2HPO_4 \cdot 12H_2O$	15 min	7,220	96	Liu et al. (2008)
Rare-earth wastewater	8.5–9.5	–	Brucite	85% H_3PO_4	30 min	4,535	93–95	Huang et al. (2011a, b)
	9.2	2.2:1:2	$MgCl_2 \cdot 6H_2O$	$Na_2HPO_4 \cdot 12H_2O$	20 min	4,420	67	Wang et al. (2012)
Calf manure digestate	9.0	1.3:1:1.3	Bittern	Bone-meal waste	15 min	1,060 ± 200	90	Siciliano and De Rosa (2014)
Coking wastewater	9.5	1:1:1	$MgCl_2 \cdot 6H_2O$	$Na_2HPO_4 \cdot 12H_2O$	30 min	500	88	Zhang et al. (2009a, b)

*Mixing time only, **The original pH value of leachate

Table 9.3 Properties of struvite

Property	Description
Chemical name	Magnesium ammonium phosphate hexahydrate
Chemical formula	$MgNH_4PO_4 \cdot 6H_2O$
Colour	White, yellow, brownish or light grey, based on crystallization media (Lee et al. 2009)
Molecular weight	245.43 g/mol
Specific gravity	1.711 (Borgerding 1972)
Solubility	Low in neutral/alkaline medium (0.018 g/100 ml at 25 °C). High in acids (0.178 g/100 ml at 25 °C in 0.01 N HCl)

9.3.2 *Struvite Formation*

The general equation of struvite formation is as given in Eq. (9.1):

$$Mg^{2+}{}_{(aq)} + NH_4^+{}_{(aq)} + PO_4^{3-}{}_{(aq)} + 6H_2O \leftrightarrow MgNH_4PO_4.6H_2O_{(s)} \tag{9.1}$$

MAP precipitation reaction lowers the pH of solution due to crystallization of soluble orthophosphate ($PO_4{}^{3-}$) (Le Corre et al. 2009), which means that the dominant form of P in struvite formation reaction would be HPO_4^{2-} or $H_2PO_4^-$ instead of PO_4^{3-}, which is explained through Eqs. (9.2) and (9.3) (Wilsenach et al. 2007):

$$Mg^{2+}{}_{(aq)} + NH_4^+{}_{(aq)} + HPO_4^{2-}{}_{(aq)} + 6H_2O \leftrightarrow MgNH_4PO_4.6H_2O_{(s)} + H^+ \tag{9.2}$$

$$Mg^{2+}{}_{(aq)} + NH_4^+{}_{(aq)} + H_2PO_4^-{}_{(aq)} + 6H_2O \leftrightarrow MgNH_4PO_4.6H_2O_{(s)} + 2H^+ \tag{9.3}$$

The formation and crystallization of struvite follow two main stages, namely, nucleation and crystal growth. Nucleation is the process of crystals birth in a solution where ions combine to form embryos. In fact, this process is basically controlled by reaction kinetics. Crystal growth is the subsequent process that corresponds to the development of crystals until reaching equilibrium. Fundamentally, the final size of crystals, hence its settle ability, is governed by crystal growth rate (Le Corre et al. 2009).

9.4 Factors Influencing Struvite Recovery

The process of struvite recovery is basically affected by several factors. These include pH, molar ratio of Mg, N and P, type of reagents, temperature, reaction time, presence of foreign ions and mixing conditions.

9.4.1 pH of Solution

The percentage of struvite in precipitates is significantly influenced by the pH of reaction, as the supersaturation increases with increasing pH. Generally, struvite can be formed within pH range of 8–10. At pH greater than 10, MAP crystallization could be inhibited due to the formation of competing compounds such as $Mg_3(PO_4)_2$, $Mg(OH)_2$, and other amorphous compounds such as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) (Ryu et al. 2008).

The effect of pH on MAP recovery has been reported in many studies. Focusing on NH_4-N removal, Ryu et al. (2008) found that the optimum pH was 9.2 for semiconductor wastewater, whereas for rare-earth wastewater, Huang et al. (2011a, b) obtained the best NH_4^+ removal within pH 8.5–9.5. For LL, a wide range of optimum pH has been found through several studies, as demonstrated in Table 9.2. Apparently, different types of pollutants present in LL could affect the optimum pH value (Zhang et al. 2012). On the opposite side, other researchers found that high-purity struvite could not be produced at pH higher than 8. This is possible because of the formation of $Mg_3(PO_4)_2$ and $Ca_3(PO_4)_2$ instead of the targeted struvite (Hao et al. 2008). In fact, the large differences between wastewaters' characteristics, as well as the possibility of ammonia to volatile at higher pH values, cause inconsistency between studies' results. Therefore, pH value should be optimized to ensure high NH_4-N removal with significant production of pure struvite.

9.4.2 Molar Ratios (Mg:N and P:N)

Theoretically, struvite is formed at the stoichiometric molar ration of Mg:N:P (1:1:1) (Eqs. 9.1, 9.2, 9.3). However, the practical ratios are usually different from the theoretical one, because of the presence of other species that form other by-products (Siciliano and De Rosa 2014; Zhang et al. 2009a, b). In that case, the optimum molar ratio should be determined for each individual case to achieve the highest efficiency of MAP recovery, as well as the least cost. Some previous studies have shown that when increasing Mg:N and/or P:N molar ratios, removal of NH_4-N could be improved (Akkaya et al. 2010; Li et al. 2012). While a number of research works have stated that increasing PO_4^{3-} dosage could enhance NH_4-N recovery more than increasing the dosage of Mg^{2+} (Ryu et al. 2008; Zhou and Wu 2012), the opposite has been observed by others (Li et al. 2012; A. Siciliano et al. 2013). This disagreement is due to the different characteristics of wastewaters and different experimental setup. Although increasing PO_4^{3-} dosage could be more valuable in some cases, achieving further removal of NH_4-N by overdosing Mg^{2+} is more preferable. Increasing the dosage of PO_4^{3-} salts could produce unwanted compounds in the final effluents, which means that additional treatment is required (Wang et al. 2006; Zhou and Wu 2012).

9.4.3 Reaction Time

Basically, struvite crystallization reaction can be accomplished rapidly. In fact, the increase in reaction time could only enlarge the size of MAP crystals; however, the development of actual struvite production would be negligible (Le Corre et al. 2009). A study conducted by Wang et al. (2012) demonstrated that the practical reaction time for effective MAP crystallization is around 20 min only. Reaction completion time could be affected by other factors. For instance, Hao et al. (2013) needed three months to obtain high-purity struvite (99.7%) at pH 7.0 (neutral) and ambient temperature (25–30 °C). However, by raising the solution's pH, reaction time could be reduced to hours or minutes, which enhances the applicability of the process. Other factors may also affect reaction time such as mixing intensity (*G* value) and reactivity of Mg and P reagents.

9.4.3.1 Temperature

Controlling temperature in MAP crystallizers ensures the purity of the recovered struvite. Practically, ambient temperatures are suitable for a thermally stable process of struvite crystallization. On the other side, high temperatures significantly affect the crystal phase and structure. Few studies illustrated the effect of temperature on struvite recovery process. Bhuiyan et al. (2008) tested the effect of temperature on the structure of struvite crystals. The authors found that thermal decomposition of MAP starts at 55 °C, and MAP phase was transformed into other phases such as bobierrite, newberyite and amorphous $MgHPO_4$. This is because solubility of struvite increases with increasing temperature (Le Corre et al. 2009). Accordingly, struvite should be dried at a temperature between 30 and 45 °C to avoid mass loss and phase transformation. These aspects should be carefully controlled during the design of struvite reactors in order to achieve higher efficiency of struvite recovery.

9.4.3.2 Magnesium and Phosphorus Sources

In general, the applied sources of Mg and P considerably influence the time required to complete the precipitation reaction, the quality of effluent, struvite purity, and the overall cost of the treatment process. Numerous types of Mg and P sources have been investigated including analytical-grade reagents and other alternatives.

9.4.3.3 Magnesium Sources

Magnesium salts were found to possess different reaction completion-time periods. By applying magnesium chloride ($MgCl_2 \cdot 6H_2O$), struvite reaction could be completed in 50 s only (Zhang et al. 2009a, b), while it takes more than 30 min to complete the reaction if magnesium oxide (MgO) is used (Li et al. 2012). Fundamentally, this distinction is referred to different solubility of the reagents; Mg or P salts with high solubility rates result in higher ionic concentrations of Mg^{2+} or $HPO_4{}^{2-}$ in solution and, therefore, improve struvite formation.

Considering the environmental aspect, applying $MgCl_2$ and magnesium sulphate ($MgSO_4$) may not be preferred, as they raise the salts concentration in the final effluents (Li et al. 2012). High salinity is a strong inhibitor for microbial activity in the following biological treatment process. In addition, $MgCl_2$ and $MgSO_4$ are costly materials, which make them economically unfeasible for large-scale applications.

MgO is one of the cheapest sources of Mg, which has been utilized in MAP technology (Li et al. 2012; Wilsenach et al. 2007). Practically, the application of MgO minimizes the salinity and electrical conductivity of the treated effluent, as MgO does not add any extra anions (Li et al. 2012). However, the sole drawback of MgO is its low solubility in water, which leads to longer reaction time periods.

Several studies have proposed different approaches to achieve an efficient application of MgO, including utilizing MgO as a dual function reagent (i.e. Mg^{2+} source and pH adjustment agent) (Huang et al. 2014) and the use of "magnesia suspension" (prepared by mixing pure MgO with de-ionized water, with a concentration of 100 g/L) (Capdevielle et al. 2013). These strategies contributed to significant cost reductions. In addition, the efficiency of MgO could be improved by dissolving MgO in an acidic media (Table 9.4). If phosphoric acid (H_3PO_4) was used for this mission, this approach could be competent. In the same context, instead of using pure Mg salts, several alternatives can be effectively employed as low-cost sources of Mg, which achieved outstanding levels of $NH_4{-}N$ recovery (Huang et al. 2014; Liu et al. 2013a, b).

The feasibility of using such alternative depends on several issues including abundance, quality and pre-treatment of the source, if required. Seawater bittern, RO and NF brines, and low-grade MgO are waste materials, which contain different amounts of Mg. Among Mg alternatives, seawater bittern contains the highest concentration of Mg^{2+}, which significantly promotes $NH_4{-}N$ recovery by MAP crystallization. Natural Mg rocks such as magnesite ($MgCO_3$) (Gunay et al. 2008b) and brucite ($Mg(OH)_2$) (Huang et al. 2011a, b) have low reactivity in wastewater. However, they are good options in places where they are abundant. To overcome the low reactivity obstacle, these rocks should be dissolved by acids to increase their reactivity, otherwise their dose needs to be increased.

Table 9.4 Alternative sources of Mg applied for MAP recovery

Mg alternative		Wastewater type	Reference
Source	Mg content		
Seawater	1,248 mg/L	Source-separated urine	Liu et al. (2013a, b)
Brine*	2,374 mg/L		
NF brine†	8,000 mg/L	Municipal-wastewater sludge centrifuge supernatant	Lahav et al. (2013)
Seawater bittern	27.5 g/L	Source-separated urine	Etter et al. (2011)
	32 g/L	Adjusted biologically treated wastewater	Lee et al. (2003)
	69 g/L*	Calf manure digestate	Siciliano and De Rosa (2014)
	48.5 g/L	Landfill leachate	Siciliano et al. (2013)
	9.22 g/L		Li and Zhao (2002)
	24.9 g/L		
Technical-grade MgO	84–86%	Industrial wastewater	Borojovich et al. (2010)
Natural magnesite ($MgCO_3$)	98% as $MgCO_3$	Landfill leachate	Gunay et al. (2008b)
Magnesite calcination by-product	53% as $MgCO_3$		Huang et al. (2014)
Natural brucite ($Mg(OH)_2$)	65% as MgO	Rare-earth wastewater	Huang et al. (2011a, b)

*Prepared synthetically, †Real nanofiltration brine

9.4.3.4 Phosphorus Sources

Phosphorus is a non-renewable source that is mainly mined from phosphate ores (apatites). Different types of P reagents have been applied as reported in the literatures. For the recovery of MAP from LL, different P salts were used such as disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$), sodium dihydrogen phosphate (NaH_2PO_4) and potassium hydrogen phosphate (K_2HPO_4). In parallel with the high solubility of these reagents, they do not produce any side elements or compounds that may negatively affect the MAP reaction. As both $Na_2HPO_4 \cdot 2H_2O$ and NaH_2PO_4 have similar solubilities, there is no significant difference between them in MAP reaction. In fact, the two reagents were used in literature and showed high efficiency. On the other hand, because of the high cost of chemical reagents, they were mostly used in lab-scale studies. Besides, phosphoric acid (H_3PO_4) was applied as a P reagent, which had benefited in improving the solubility of Mg reagent ($MgCO_3$) (Gunay et al. 2008b) and reducing the chemical cost (Di Iaconi et al. 2010).

Generally, the cost of struvite recovery is mainly contributed by the consumption of P salts (Siciliano et al. 2013), which are basically produced from the apatite's ores that are estimated to last about 50–100 years (Driver et al. (1999). However, despite the scarcity and the high cost of P sources, only a few studies have been conducted to find alternative sources of P to date. Bone-meal waste (a by-product of meat-waste thermal treatment, 61.6% PO_4^{3-}) was utilized as a low-cost source of P by Siciliano et al. (2013) and Siciliano and De Rosa (2014) for NH_4-N recovery in LL and calf manure digestates, respectively. Additionally, Huang et al. (2014) applied wasted H_3PO_4 to treat NH_4-N in LL. In these studies, high efficiencies of NH_4-N recovery were achieved, relatively similar to those achieved when pure chemicals were used. Recently, using fish waste bone ash as an alternative source of P has demonstrated considerable recovery of NH_4-N (≈90%) in synthetic solution (Darwish et al. (2017). Practically, P can be found in many kinds of waste materials such as food waste and animals waste bones. In addition to the economic benefit that could be derived from struvite recovery, using alternative Mg and P sources contributes to waste minimization and utilization simultaneously, hence enhances the sustainability of struvite recovery process.

9.4.3.5 Effect of Foreign Ions

The process of NH_4-N recovery via MAP precipitation is highly competed due to the presence of different sorts of foreign ions. Previous literatures showed that calcium (Ca^{2+}) is one of the main elements that hinders MAP formation, because Ca^{2+} has high potential to compete with Mg^{2+}, forming amorphous calcium phosphates ($Ca_3(PO_4)_2$). This process is controlled by pH and initial concentrations of P, N and Mg (Ca/Mg ratio) in wastewater. In general, molar ratios of Ca:Mg higher than 0.5 were noticed to enhance the reaction between Ca and PO_4 ions, hence lowering the efficiency of NH_4-N recovery (Lee et al. 2013; Song et al. 2011). Huang et al. (2011a, b) depicted that when the molar ratio of Ca:Mg was raised from 0 to 0.75 (Mg = 985 mg/L), NH_4-N recovery was drastically decreased from 88 to 58% (initial NH_4 = 985 mg/L).

Based on the reported characteristics of LL in literature, it was found that LL usually contains low amounts of Ca such that when struvite precipitation is applied, the molar ratio of Ca:Mg becomes less than 0.1 (Huang et al. 2014; Siciliano et al. 2013). Therefore, Ca is not considered as a major problem when recovering NH_4-N from LL by struvite precipitation.

High concentrations of K can be found in different types of wastewater such as human urine (Liu et al. 2008). In previous work, K was shown to co-precipitate in parallel with struvite crystals, especially at low NH_4-N concentrations, forming $MgKPO_4.6H_2O$ (MPP), which is called "Struvite-K" or "Potassium-struvite" (Di Iaconi et al. 2010; Wilsenach et al. 2007). Struvite-K is one of the struvite analogues and the behaviour of the formation could be explained by Eq. (9.4) (Wilsenach et al. 2007).

$$Mg^{2+}{}_{(aq)} + HPO_4^{3-}{}_{(aq)} + K^+{}_{(aq)} \rightarrow MgKPO_{4(s)} + H^+ \quad (9.4)$$

However, the presence of K causes less interference with NH_4-N recovery than that of Ca. According to Huang et al. (2011a, b), a slight drop in NH_4-N removal from synthetic piggery wastewater was observed when K:Mg molar ratio was increased from 0 to 0.75. Besides, the effect of K was illustrated in previous researches, which targeted the recovery of K from source-separated urine in the form of MPP (K-struvite). Wilsenach et al. (2007) reported that MAP has more tendency to be formed than MPP. In the same context, for P and K recovery from synthetic urine, it was demonstrated that, when NH_4-N concentration was increased from 0 to 500 mg/L, the efficiency of MPP precipitation, as well as NH_4-N removal, was decreased from 77% to 65% and from 97% to 77%, respectively, ($K_0 = 52.4$ mM/L, pH 10, Mg:K:P = 2:1:2) (Xu et al. 2011). These studies indicated that the effect of K is less significant than Ca; however, it should be taken into consideration in the cases of high concentrations of K, such as those reported for LL (Di Iaconi et al. 2010; Huang et al. 2014 and Kochany and Lipczynska-Kochany 2009).

Wastewater may contain high concentrations of heavy metals that could affect the purity of struvite. For agronomic field applications, the incorporation of heavy metals, such as chromium (Cr), is a vital issue if struvite is to be used as a fertilizer (Rouff 2012a, b). Minute levels of some heavy metals, such as arsenic (As) in urine (Ronteltap et al. 2007) and sewage sludge effluent (Uysal et al. 2010), were found co-precipitated with struvite. Liu et al. (2013a, b) found a lower limit of heavy metals in struvite obtained from swine wastewater. The authors reported that this struvite is safer compared to struvite recovered from LL or some other industrial wastewaters. However, for sustainable agriculture, the potential accumulation of heavy metals in soil and plants is undesirable as heavy metals may consequently cause hazardous effects for humans and animals. Table 9.5 shows the effect of some heavy metals on MAP recovery. Heavy metals have shown two sorption mechanisms during MAP crystallization, i.e. co-precipitation and adsorption. Co-precipitation was found to be the sorption mechanism of As(V) (Ma and Rouff 2012) and Al^{3+} (Acelas et al. 2015), while other metals like Cu^{2+} and Zn^{2+} (Muryanto and Bayuseno 2014), and As(III) (Ma and Rouff 2012) were found to be adsorbed on struvite surface. Table 9.5 shows that some heavy metals such as aluminium (Al^{3+}), copper (Cu^{2+}) and zinc (Zn^{2+}), which are highly concentrated in swine wastewater and sludge digestion liquors, have the ability to cause inhibition on struvite formation (Acelas et al. 2015; Liu et al. 2011a, b), while the sorption of either Cr(III) or Cr(VI) on struvite has several environmental implications related to agriculture sustainability and human health. The effects of many other heavy metals (e.g. Ni and Cd) were not investigated in literature, although high concentrations are commonly found in different types of wastewater such as LL.

9.4.3.6 Mixing Conditions

Struvite crystallization (nuclei generation and growth) is significantly influenced by mixing intensity value (G value) and mixing duration (t_d). While the generation of crystal nuclei depends on the initial value of G, crystals' growth relies on t_d

Table 9.5 Effect of heavy metals on MAP recovery

<table>
<tr><th>Heavy metal</th><th colspan="2">Potential effect</th><th>Reference</th></tr>
<tr><td>Al^{3+}</td><td colspan="2">Completely inhibited struvite formation even at low molar ratio of Mg^{2+}:Al^{3+} (2:1)</td><td>Acelas et al. (2015)</td></tr>
<tr><td>As(V)</td><td colspan="2">Co-precipitation (substitution of $AsO_4{}^{3-}$ for $PO_4{}^{3-}$ forming $MgNH_4AsO_4 \cdot 6H_2O$). Highest rate at pH 10</td><td rowspan="2">Ma and Rouff (2012)</td></tr>
<tr><td>As(III)</td><td colspan="2">Adsorption on struvite surface. Lower sorption affinity than As(V)</td></tr>
<tr><td>Cr(VI)</td><td>Lower sorption affinity than Cr(III)
Toxic to plants even at low concentrations</td><td rowspan="2">– Affect MAP structure stability
– Increase nutrients leaching rate</td><td rowspan="2">Rouff (2012a, b)
Rouff (2012a, b)</td></tr>
<tr><td>Cr(III)</td><td>Toxicity increase with concentration</td></tr>
<tr><td>Cu^{2+} and Zn^{2+}</td><td colspan="2">Adsorption of ions on struvite surface. Trace amounts (<5 mg/L) could considerably retard struvite crystallization rate with a maximum percentage of 25%</td><td>Muryanto and Bayuseno (2014)</td></tr>
</table>

(Le Corre et al. 2009). Wang et al. (2006) studied the effect of mixing intensity on struvite crystals, and showed that a G value higher than 76 s^{-1} could result with break down of large crystals, hence reducing crystal growth and settleability of the crystals. In addition, Usyal and Kuru (2013) showed that struvite crystals appeared with irregular shape when mixing speed was higher than 260 rpm. Besides, Kim et al. (2009) reported significant improvement in the removal efficiencies of $NH_4{-}N$ and $PO_4{-}P$ by increasing both G value and t_d. However, as noticed from Tables 9.5 and 9.6, the studies that have investigated the effect of G, or mixing speed, on struvite crystallization were carried out through lab-scale experiments. Thus, this effect should be studied in larger scale researches to have more in-depth understanding.

In their pilot-plant, Pastor et al. (2008) studied the effect of mixing duration on struvite recovery. They found that low duration (<2 h) could increase the percentage of fine struvite crystals (10–100 μm) in the precipitates, which reduced settling efficiency and promotes the wash-out of crystals. Therefore, mixing conditions should be managed wisely to achieve the highest $NH_4{-}N$ recovery with the greatest purity of struvite.

Table 9.6 Effect of mixing intensity/speed on MAP recovery

Wastewater type (V_{sample})	Mixing speed/intensity	NH_4-N (mg/L)		PO_4-P (mg/L)		Reference
		Initial	Removal	Initial	Removal (%)	
Synthetic piggery wastewater (2 L)	76 s^{-1}	420	N.S.*	2.9	91.9	Wang et al. (2006)
Semiconductor wastewater (2 L)	$G \cdot t_d > 10^5$	4	86%	99	70	Kim et al. (2009)
		10	88%			
		79	98%			
Anaerobic effluent of dairy processing industry (100 mL)	260 rpm	45	88.4%	0.36	79.3	Usyal and Kuru (2013)

*N.S.: Not studied

Table 9.7 Reduction of other pollution parameters during MAP recovery

Wastewater type	Reduction of pollution parameters (%)					References
	COD	SS	TOC	Colour	Turbidity	
Landfill leachate	80	N.S.	N.S.	N.S.	N.S.	Kabdaşli et al. (2000)
	25.7	N.S.	N.S.	22.5	36.4	Akkaya et al. (2010)
	N.S.	N.S.	N.S.	43	62	Gunay et al. (2008a)
	10	80	N.S.	N.S.	50	Gunay et al. (2008b)
	5	N.S.	N.S.	N.S.	N.S.	Siciliano et al. (2013)
Semiconductor wastewater	81, 66*	N.S.	N.S.	N.S.	N.S.	Ryu et al. (2008)
Poultry manure wastewater	50	N.S.	N.S.	49.8	N.S.	Yetilmezsoy and Sapci-Zengin (2009)
Swine wastewater	22.1	N.S.	20.7	N.S.	N.S.	Zhang et al. (2012)
Dairy processing wastewater	68.4	N.S.	N.S.	N.S.	N.S.	Usyal and Kuru (2013)

*Total COD (81%), Soluble COD (66%). N.S.: Not studied

9.4.4 Contribution of Struvite Precipitation for Reduction of Pollutants

Struvite precipitation process has some side contribution in the reduction of other pollutants, particularly organic matter and total suspended solids (TSS). Table 9.7 shows the main parameters that were reduced during MAP precipitation. The decrease in

COD concentration during MAP precipitation was attributed to co-precipitation of other compounds alongside with struvite (Yetilmezsoy and Sapci-Zengin 2009). In fact, the excessive dosages of Mg reagents contribute to more removal of COD, SS, colour and turbidity, as Mg ions have similar behaviour to flocculants, which remove particulate organic matter (Akkaya et al. 2010; Ryu et al. 2008).

Moreover, adsorption of some organic substances onto the surface of struvite improves COD reduction. A similar removal trend of organics, expressed as TC and TOC, was reported by Zhang et al. (2012), but with less removal percentage as compared to COD. The removal of other pollutants during struvite crystallization was found to be influenced by pH, Mg and P dosages and mixing intensity (Akkaya et al. 2010; Gunay et al. 2008a).

High concentrations of some pollutants could have negative impact on NH_4-N recovery. Barnes et al. (2007) found that NH_4-N removal from a mature LL was 98% when initial TSS was 320 mg/L. However, the removals were reduced to 78% and 71% when initial TSS concentrations were 3,600 and 5,160 mg/L, respectively. On the contrary, Tarragó et al. (2018) confirmed that high concentrations of TSS (1–3 g-TSS/L) in digested swine manure have favoured the agglomeration of struvite fine crystals, thus obtaining larger crystalline structure.

The removal of some pollutants could be affected by the type of Mg reagent. As reported by Yetilmezsoy and Sapci-Zengin (2009), using MgO as Mg source has resulted in lower COD and colour reduction than that of $MgCl_2$ or $MgSO_4$. This could be due to the vast difference between the solubility of Mg reagents in wastewater. The high reactivity of Mg reagent (e.g. $MgCl_2 \cdot 6H_2O$) contributes to higher rate of MAP formation, in parallel with higher coagulation effect, which is the main reason for COD and colour reduction. On the other hand, the opposite may occur when using Mg reagent with low reactivity (e.g. MgO), leading to lesser reductions in COD and colour.

9.5 Integration of MAP Precipitation Technology with Other Treatment Techniques

The integration of struvite technology with other treatment processes has been shown to be useful for highly polluted wastewaters. As recommended by Pal and Kumar (2014), this kind of integration promotes the sustainability of wastewater treatment, owing to the substantial economic advantages that could be obtained. As a pre-treatment process, MAP precipitation was found to be an effective solution for high NH_4-N levels that inhibit the bacterial activity in biological treatment stages (Chen et al. 2013; Jaafarzadeh et al. 2010). Additionally, struvite precipitation could be applied as a post-treatment process to enhance the final effluent quality in matching with the discharge limits. For example, Akkaya et al. (2010) achieved only 7.9% NH_4-N removal during anaerobic treatment, whereas the efficiency was increased to 98% after applying struvite precipitation as a post-treatment process. Moreover, as reported by Siciliano et al. (2013), utilizing alternative P-source (P-rich acidic solution) has shown a significant improvement in the peroxide-oxidation treatment for COD and NH_4-N removal from landfill leachate.

9.5.1 *Struvite as a Fertilizer*

In order to attain sustainable agricultural practices, safe fertilizers have to be applied to avoid harmful effects on plants, humans and surrounding environment. Struvite has a significant role in the agricultural field. It is an eco-friendly and controlled fertilizer as its nutrients' releasing rate is slow. These characteristics make struvite a more preferred fertilizer for different kinds of plants and crops, such as wheat (Degryse et al. 2016), triticale, oats and alfalfa (Hilt et al. 2016), maize and tomato (Uysal et al. 2014) and Chinese cabbage (Ryu et al. 2012). Thorough details on the role of struvite in agronomic field are presented in Mukhlesur Rahman et al. (2014) and Yetilmezsoy et al. (2017).

9.5.2 *Cost Reduction Opportunities*

In several types of wastewater, the concentrations of P and Mg are relatively low compared to NH_4-N, hence large amounts of reagents are required to recover struvite. As a result, more cost is added to the process. In most pilot and large-scale applications, struvite recovery applied on P-rich wastewaters did not require any P-addition (Pastor et al. 2008; Suzuki et al. 2007). However, similar applications for NH_4-N recovery from NH_4-N-rich wastewaters but poor in P, such as LL (Li et al. 2012; Xiu-Fen et al. 2011), would be more costly as both Mg and P additions are needed. Siciliano et al. (2013) reported that the cost of P reagents is a limiting factor that influences the economic feasibility of struvite recovery. Therefore, the main target of MAP technology, especially if applied as a pre-treatment process, should not be the recovery of all NH_4-N in the wastewater in order to avoid the high consumption of Mg and P reagents. The following are suggested approaches to reduce the cost of NH_4-N recovery through struvite technology.

9.6 Struvite as Fertilizer in Agriculture

A number of studies have shown that applying struvite-fertilizer resulted with higher nutrients uptake than other types of fertilizers (Uysal et al. 2014) as well as lower heavy metals accumulation (Ryu et al. 2012). Precisely, the slow-releasing property of MAP provides a controlled supply of nutrients for a longer period, which ensure maximum N uptake by plants and minimum N loss. Application of MAP fertilizer would also reduce greenhouse gas emission, mainly due to the gradual release of nutrients. Rahman et al. (2011) found that leaching loss of N from MAP-treated soil (1.98–2.05%) was lower than that from urea-treated soil (6.47–7.82%). The leaching rate of P was also very low for both soils, hence, making MAP an efficient and eco-friendly N fertilizer.

9.6.1 Low-Cost Sources of Magnesium and Phosphorus in Struvite Crystallization Process

Several research works have evaluated the feasibility of utilizing low-cost sources of Mg and/or P in order to reduce the recovery cost. The cost of struvite recovery from LL using conventional sources of Mg and P (MgO and H_3PO_4) is approximately \$24–28/m^3 leachate (Di Iaconi et al. 2010; Gunay et al. 2008b). Gunay et al. (2008b) reported that using natural magnesium carbonate ($MgCO_3$) saved nearly 18% of struvite recovery cost (from \$28 to 22/m^3 leachate). However, a better cost saving could be achieved by applying low-cost sources of Mg and P together. As illustrated by a few studies, the use of by-products of Mg and P together could reduce the costs of struvite recovery by 43–68% (Huang et al. 2014; Siciliano and De Rosa 2014; Siciliano et al. 2013). Hence, more studies are required to explore other alternative sources of P, particularly such as those from food industries.

9.6.2 Reuse as Source of Magnesium and Phosphorus

The main idea of reusing struvite is based on eliminating NH_4 from struvite matrix and reproducing Mg and P compounds that can be usable for $NH_4{-}N$ recovery. Five main techniques have been recently studied for MAP reuse, namely pyrogenation (heating under alkali conditions), distillation with alkaline addition, electrolysis, breakpoint chlorination and microwave decomposition. The procedures and related details have been thoroughly discussed elsewhere (Liu et al. 2013a, b; Türker and Çelen 2007). Researches on pyrogenation demonstrated that such technology could reduce the chemicals costs by 67% (Huang et al. 2016a, b). Later, Huang et al. (2016a, b) developed a new pyrogenation process that used low temperature and negative pressure. The authors were able to save around 86% of treatment cost, compared to the cost of using pure chemicals without recycling. By applying distillation with caustic addition, Türker and Çelen (2007) reached 100% vaporization of NH_3 and achieved around 41.6% cost reduction. Electrolysis technique was investigated by Liu et al. (2011a, b), at which the researchers achieved more than 65% cost reduction. Recently, Huang et al. (2015) developed a novel approach of struvite recycling, based on chlorination decomposition of struvite. Using sodium hypochlorite (NaClO), 98%

of $NH_4{-}N$ was removed at pH 6 and Cl/$NH_4{-}N$ wt. ratio of 8.2:1. Additionally, more than 92% of $NH_4{-}N$ in LL could be removed using the recycled product, in which nearby 34% of the chemical cost could be saved. Siciliano et al. (2016) tested the effectiveness of struvite residues obtained through acid decomposition, using different kinds of acids. The authors proved that hydrochloric (HCl) and acetic acid (CH_3COOH) were highly effective in releasing ammonium at only 50 °C and pH 5.5. Besides, they reported a cost saving of nearly 74% after reusing the decomposition residues.

From a practical point of view, the applicability of those approaches is somehow different from one case to another based on different aspects, such as the initial concentration of pollutants ($NH_4{-}N$, P, Mg and heavy metals) and the applied reagents. Moreover, the energy and manpower costs are rarely considered in the studies. The marketing potentiality of struvite as a fertilizer is a vital issue that has to be counted, because struvite is a slow-releasing fertilizer, which cannot be applied for all types of plants. If the cost of struvite production is very high, the fertilizing industry would prefer other kinds of P fertilizers that give the same fertilization efficiency as struvite, such as monocalcium phosphate ($Ca(H_2PO_4)_2 \cdot H_2O$)) and dicalcium phosphate ($CaHPO_4 \cdot 2H_2O$) (Hao et al. 2013). Furthermore, all related investment and running costs should be studied carefully for each individual case, to be able to achieve a complete economic feasibility assessment of the final product (Yetilmezsoy et al. 2017).

9.6.3 Research Need and the Way Forward

Considering the aspects mentioned in the previous sections, several research needs should be highlighted. In general, a low-cost process of $NH_4{-}N$ recovery is one of the main challenges that face the researchers in MAP technology. Applying the waste bone ash, together with MgO, could be an efficient alternative replacing pure chemicals. In fact, such combination has not been investigated before. Looking at the effect of foreign ions, it seems that the interaction effect of pH, Mg:N molar ratio, K and G value is not clear. Essentially, there is a need to understand more of this aspect, to facilitate the application of MAP technologies in high-K wastewaters. Besides, as wastewater contains high loads of organics, the influence of the individual organic substances should be evaluated. Furthermore, some industrial wastewaters contain high concentrations of heavy metals. Thus, the investigation of the potential effect and sorption mechanism of these heavy metals is important, as heavy metals have the ability to be included in MAP structure.

9.7 Conclusion

Nitrogen is one of the main pollutants in landfill leachate, which is also considered as a valuable element. Struvite crystallization technology is proved to be an effective approach to recover nitrogen in the form of eco-friendly fertilizer. This chapter showed that up to 98% of nitrogen in landfill leachate could be recovered as struvite. However, this is governed by several factors such as pH, Mg:N:P molar ratio, types of Mg and P reagents and other experimental conditions. In general, the major challenges to achieve a sustainable struvite technology, integrated with other treatment processes, are cost-effectiveness and purity enhancement. These aspects should be considered in future research to support the sustainability of MAP technology and improve its applicability in landfill leachate treatment.

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Chapter 10
Qualitative Characterization of Healthcare Wastes

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Abstract The biological hazard inherent in the clinical wastes should be considered during the management and treatment process as well as the disposal into the environment. In this chapter, the risks associated with the clinical wastes as well as the management of these wastes are discussed. The chapter focused on reviewing the types of healthcare wastes generated from hospitals and clinics as well as the regulations and management practices used for these wastes. Moreover, the health risk associated with the infectious agents which have the potential to be transmitted into the environment. It has appeared that the clinical wastes represent real hazards for the human health and the environment if they were not managed properly.

10.1 Introduction

Healthcare wastes are general terms used to define the wastes containing blood and infectious agents which are generated from healthcare facilities. In some references, these wastes are defined as clinical wastes, medical waste, biomedical wastes, hospital wastes, healthcare waste, infectious waste, and hazard and biohazard wastes. However, this definition excludes the solid wastes which can be recycled or reused even after the treatment process (Noman et al. 2018a, b). Moritz (1995) suggested that the disposable or waste terms should be used for non-recyclable materials, while

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A. A. S. Al-Gheethi et al. (eds.), *Prospects of Fresh Market Wastes Management in Developing Countries*, Water Science and Technology Library 92,
https://doi.org/10.1007/978-3-030-42641-5_10

the term of reusable or recyclable is used to define the solid materials which might be reused or recycle. Based on this definition the sharps, cotton, and medical devices are defined as a recyclable materials, while blood, body parts, chemicals, pharmaceuticals, and radioactive materials are wastes. In other meaning, it has to be mentioned that not all the wastes generated from the hospital are classified as clinical wastes. According to the Controlled Waste Regulations 1992 (S1588), the clinical wastes are defined as any waste with human or animal tissue, excretions, blood as well as body fluids, excretions, swabs, syringes, and needles. These wastes are generated from the medical, dental, nursing, veterinary pharmaceutical or similar practice, investigation, treatment, care, teaching, or research centers. Figure 10.1 depicted the recyclable materials and clinical wastes generated from the healthcare facilities.

The clinical waste is classified as a type of the biohazard waste as it is heavily loaded with infectious agents which included Staphylococcus *aureus*, pathogenic strains of *E. coli*, *Salmonella* spp. *P. aeruginosa, Enterococcus faecalis, Klebsiella pneumonia* as well as opportunistic fungi such as *Aspergillus niger*, *A. fumigatus*, *A. tubingensis*, *A. terreus var. terreus,* and *C. lunata* (Banana 2013; Noman et al. 2016a, b; Efaq et al. 2017). In a view for the health risk associated with the presence of infectious agents in the clinical wastes, WHO (2005) classified the pathogens

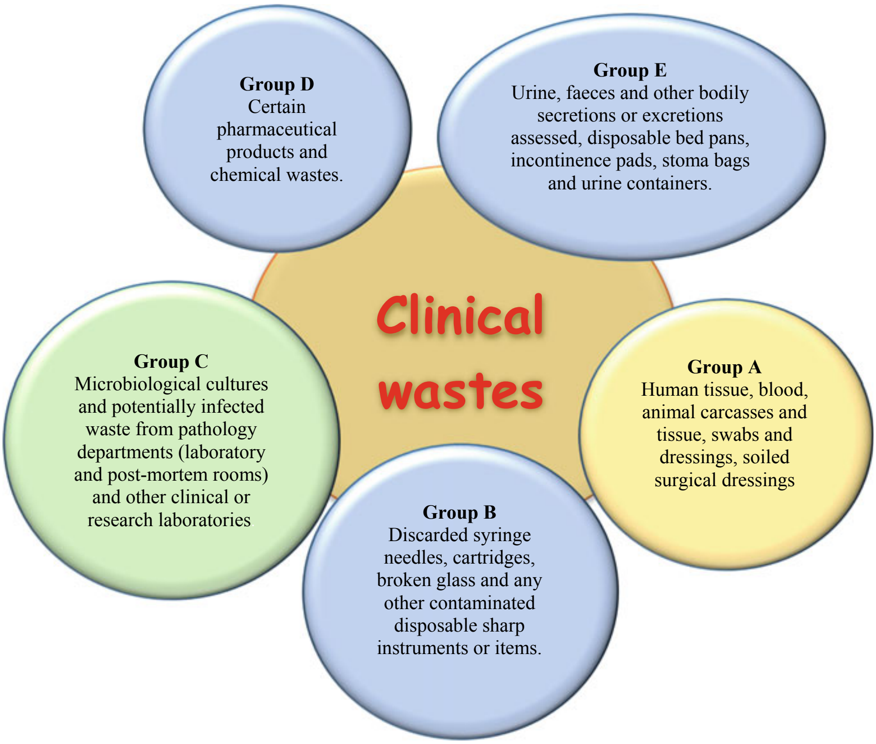

Fig. 10.1 Clinical wastes classes generated from the healthcare facilities

in the microbiological laboratories based on pathogenicity into four risk classes, where *E. coli* and *Salmonella* spp. are classified within Risk Group 2 (moderate individual risk, low community risk) and Risk Group 3 (high individual risk, low community risk), while *P. aeruginosa, E. faecalis, K. pneumonia,* and *S. aureus* are within Risk Group 2. Subsequently, it can be noted that these wastes lay between the non-hazardous wastes which have microbial loads that belong to the Risk Group 1 (no or low individual and community risk) and high hazardous wastes which contain infectious agents within Risk Group 4 (high individual and community risk).

Human body fluids (HBFs) are among the various types of the clinical wastes which have high potential source for surviving infectious agents due to the presence of the survival factors such as nutrients and growth factors available in the blood (Pruss et al. 1999). Moreover, blood has high nutritive value and can be considered as enriched medium for bacterial and fungal growth (Carrwtero and Pares 2000). In this paper, the quantities and qualities of the clinical wastes as well as the health risk associated with these wastes in the developing countries are reviewed.

10.2 Clinical Waste Generation

The amount of clinical waste generated from different healthcare facilities, which has been increased at a rapid pace in recent years, has drawn serious attention from the society. In Malaysia, it was estimated that the total quantities of the clinical wastes have increased from 3,303 to 18,055 tons per year during the period between 1997 and 2012. In 2010, 8000 tons was generated, while it is expected to reach 33,000 in 2020 (DS 2013; Ambali et al. 2013). Clinical wastes represent more than 50% of the total solid and liquid wastes generated from the healthcare facilities, with 10–25% of them classified as high-risk wastes (Shinee et al. 2008).

The quantities of the clinical wastes vary among countries depending on the total population and healthcare facilities as well as the size and type of the medical institution and number of patient care. In the United States of America, the quantities of the clinical wastes were estimated to be 6,600 tons of waste per day. In 2012, Malaysia had more than 398 hospitals (147 public and 251 private hospitals) with 42707 versus 14165 beds for public and private hospitals, respectively (DS 2013). Based on the daily amount of clinical wastes generated in Malaysia (1.9 kg/bed/day), the estimated quantities of clinical wastes generated from public hospitals in 2012 were 29,617.546 tons/year. However, DS (2013) reported that only 18,055 tons have been handled for destruction by incinerator. It was due to that the maximum loading capacity for incinerators in Malaysia is 18,000 tons/year (Frost and Sullivan 2010). In comparison, Yemen has only 56 hospitals which generate different clinical wastes (MPHP 2012). The quantities of the clinical wastes are between 7000 and 10,000 tons/year.

The studies indicated that the quantities of the clinical wastes generated from the developed countries are more than that for developing countries (Fig. 10.2). For instance, in North America, 5.5 kg/bed/day are generated compared to

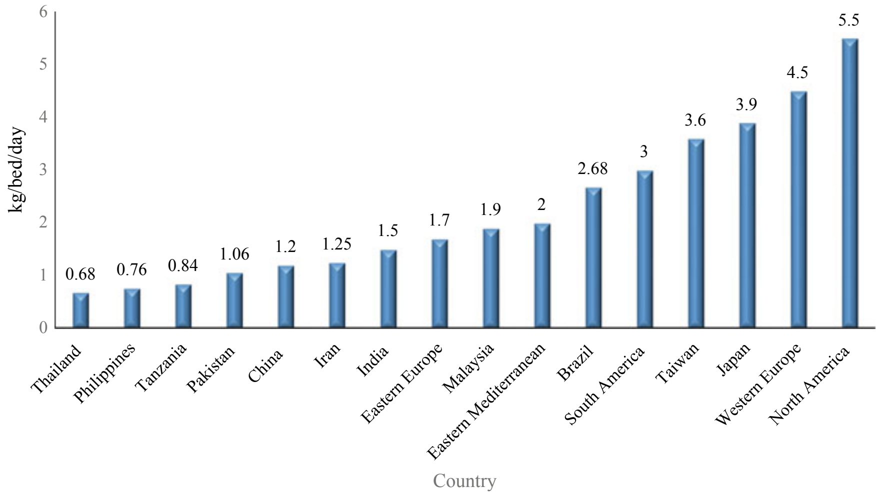

Fig. 10.2 Estimated average healthcare waste generation in different region in the world (WHO 2005; Visvanathan 2006; Johannessen 1997; De Silva et al. 2011; Cheng et al. 2009; Ruoyan et al. 2010)

0.68 kg/bed/day in Thailand. However, the average of the clinical waste quantities is between 0.5 and 3 kg/bed/day (WHO 2000). In Malaysia, the quantities of the clinical wastes generated have increased annually due to the fast increase in the total population (1.5%). The quantities of clinical wastes handled for the incineration increased by 37.1% from 11000 in 2006 to 18000 in 2016 (Fig. 10.3).

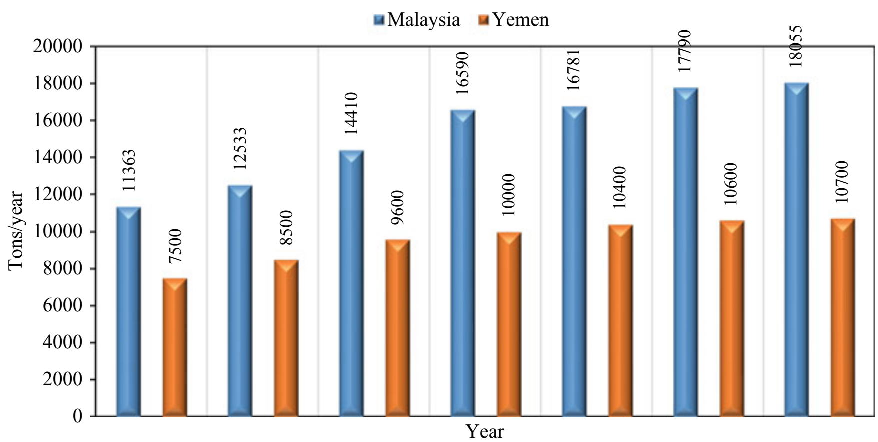

Fig. 10.3 Quantity of clinical waste for destruction at incinerator Malaysia and Yemen. *Source* Department of Environment (DS 2011, 2013; MPHP 2012)

Table 10.1 Solid and liquid clinical wastes generated from healthcare facilities

	Clinical wastes
Liquid wastes	Ascitic fluid, autoclave water, blood waste, CSF, expired fluids, pus, saliva, semen, sputum, test reagents, urine, used water
Solid wastes	Abortus, ampules, amputated body parts, biopsy tissue, blades, blend, bone marrow or lymph nodes, broken glasses, catheters, chemical wastes, excised tumors, expired drugs, feces, gloves, hair, lab cultures, mask, nails, needles, Pasteur pipette, placentae, plastic loops, polyester, polyethylene, polyurethane, respiratory secretions, scalpels, sharps, skin biopsy, skin snip, slides, spandex, specimen bottles, surgical dressings, swabs, syringes, teeth, test strips, tissue paper, tongue depressor, used cotton, used gauze, used rubber, vaginal scraping, yellow and blue tips

According to WHO (2016), 15% of the waste generated from hospitals are considered as hazardous wastes. However, Graikos et al. (2010) claimed that the hazardous clinical waste in Greece was between 13 and 92% of the total wastes. These wastes are classified by WHO into 10 categories based on their characteristics and the treatment method for each type (Table 10.1). It can be noted that the incineration is suggested for five categories including human anatomical waste, animal waste, microbiology and biotechnology waste, sharps and medicines and cytotoxic drugs, while autoclaving is suggested for the treatment of solid wastes (blood and body fluids, microbial cultures). Nonetheless, the clinical wastes are divided mainly into two classes, namely, solid and liquid wastes (Table 10.2), but the main consideration for the classification of the wastes generated from the hospitals as infectious or non-infectious waste is the presence of body fluids. There are two sources for the generation of the clinical wastes including diagnostic laboratories and the clinics (Fig. 10.2). The quantities of the wastes generated from each source are illustrated in Table 10.3. According to Cheng et al. (2010), the maximum generation of clinical wastes in Taiwan was from blood centers (3.14 kg/bed/day), private clinics (1.91 kg/bed/day), medical laboratories (1.07 kg/bed/day), and public clinics (0.053 kg/bed/day) (Fig. 10.4).

10.3 Health Risk of the Clinical Wastes

Among different types of the clinical wastes, the human body fluids (HBFs) represent the most potent source for the infectious agents. In some references, these wastes include identifiable human tissue, blood, and swabs (WHO 2005; MOH 2009). Moreover, these wastes act as a vector for transmission of pathogens from the wastes into the human and environment during the disposal process. The importance of the HBFs belongs to their composition which contains sugars, protein, fats, and starch that support the microbial survival in the hard environment (Hall 1989). The clinical wastes are rich with the nutrients and subsequently the pathogens such as bacteria, fungi, and virus; however, the potential infectious risks associated with HBFs waste management and treatment are still unknown due to the absence of critical reports on

Table 10.2 Qualities of clinical wastes generated from diagnostic labs in Yemen

Diagnostic laboratory	Clinical wastes
Microbiological lab	Autoclave wastewater, blades, blend, blood waste, broken glasses, chemical wastes, CSF, gloves, hair, lab cultures, mask, needles, Pasteur pipette, plastic loops, pus, respiratory secretions, saliva, semen, skin biopsy, skin snip, specimen bottles, sputum, swabs, syringes, tissue paper, urine, used cotton, used gauze, yellow and blue tips, vaginal scraping
Histopathological lab	Broken glasses, slides, blades, chemical wastes, excised tumors, gloves, masks, scalpels, sharps, specimen bottles, tissue paper, used cotton, used gauze
Parasitological lab	Broken glasses, chemical wastes, feces, gloves, mask, needles, Pasteur pipette, sharps, slides, specimen bottles, tissue paper, used cotton, used gauze
Urological lab	Broken glasses, chemical wastes, gloves, mask, needles, Pasteur pipette, slides, specimen, bottles, test reagents, test strips, tissue paper, urine, used cotton, used gauze
Dental labs	Blades, broken glasses, chemical wastes, gloves, mask, saliva, sharps, specimen bottles, tissue paper, used cotton, used gauze
Immunological lab	Blades, blood waste, broken glasses, chemical wastes, gloves, mask, needles, Pasteur pipette, slides, specimen bottles, syringes, test reagents, used cotton, used gauze, yellow and blue tips
Hematological lab	Blades, blood waste, bone marrow or lymph nodes, broken glasses, chemical wastes, CSF, excised tumors, gloves, mask, needles, Pasteur pipette, sharps, slides, specimen bottles, syringes, test reagents, used cotton, used gauze, yellow and blue tips
Biochemistry lab	Broken glasses, chemical wastes, CSF, gloves, masks, needles, Pasteur pipette, slides, specimen bottles, syringes, test reagents, test strips, urine, used cotton, used gauze, yellow and blue tips
Radiological lab	Radioactive wastes

the infection cases caused by the pathogens transmitted through the clinical wastes (Salkin 2003).

In a view for the microbial loads of clinical wastes which were determined by several authors in the literature, it can be indicated that the clinical wastes contain many of the pathogens such as *A. baumannii, Bacillus sp., E. coli, K. pneumonia, P. mirabilis, P. aeruginosa, S. aureus, S. epidermidis, Salmonella* spp., *Legionella, Kocuria sp., Lactobacillus sp., Micrococcus spp., Brevibacillus sp., M. oxydans, and P. acnes* (Eckmanns et al. 2008; Alagoz and Kocasoy 2008; Park et al. 2009). *E. coli* and *S. aureus* have been isolated from the clinical wastes at New Delhi hospital (Savita et al. 2004). Al-Ghamdi (2011) revealed that *Bacillus* sp. was the most predominant bacteria in the hospital environment. According to WHO (1999), human immunodeficiency virus (HIV) and hepatitis viruses B and C are among the infections which are transmitted via healthcare waste.

Table 10.3 Quality of clinical waste generated from clinics

Clinics	Clinical wastes
First aid clinics	Ampules, blades, blood waste, broken glasses, expired drugs, expired fluids, fluid nutritious, gloves, mask, needles, polyester, scalpels, sharps, surgical dressings, swabs, syringes, tissue paper, used cotton, used gauze, used rubber
Emergency	Ampules, ascitic fluid, blades, hair, mask, needles, sharps, surgical dressings, teeth, tissue paper, used cotton, used gauze
Critical care	Ampules, ascitic fluid, blades, broken glasses, catheters, gloves, mask, needles, sharps, surgical dressings, syringes, tissue paper, drugs, used cotton, used gauze, used rubber
Ear, nose, and throat clinic	Used cotton, ampules, blades, blood waste, gloves, mask, needles, sputum, pus, swabs, syringes, tongue depressor, used cotton, used gauze
Respiratory diseases clinic	Ampules, ascitic fluid, gloves, mask, respiratory secretions, saliva, sputum, swabs, syringes, tissue paper, tongue depressor
Kidney and urinary tract clinic	Ampules, gloves, catheters, drugs, polyester, polyethylene, surgical dressings, used cotton, used gauze, tissue paper
Blood diseases clinic	Ampules, blood waste, gloves, mask, needles, Pasteur pipette, surgical dressings, used cotton, used gauze
Heart diseases clinics	Ampules, catheters, gloves, mask, needles, used cotton, used gauze
Obstetrics and gynecology clinic	Abortus, ampules, blood waste, gloves, mask, placentae, surgical dressings, swabs, syringes, used cotton, used gauze
Surgical clinic	Ampules, blades, blood waste, gloves, scalpels, surgical dressings, spandex, swabs, syringes, used cotton, used gauze, chemical wastes, polyester
Internal medicine clinic	Ampules, biopsy tissue, gloves, needles, sharps
Orthopedic clinic	Ampules, blood waste, gloves, needles, scalpels, used cotton, used gauze
Eye diseases clinic	Gloves, needles, used cotton, used gauze, swabs, sharps
Dermatologist and genital clinic	Gloves, hair, mask, nails, needles, scalpels, sharps, skin biopsy, skin snip, semen, surgical dressings, swabs, syringes, vaginal scraping
Dental clinics	Teeth, gloves, needles, saliva, sharps, surgical dressings, swabs, syringes, tongue depressor
Children's clinic	CSF, gloves, needles, surgical dressings, swabs, syringes, tongue depressor
Operating room	Catheters, amputated body parts, ampules, excised tumors, ascitic fluid Blood waste, gloves, mask, needles, scalpels, sharps, surgical dressings, swabs, syringes

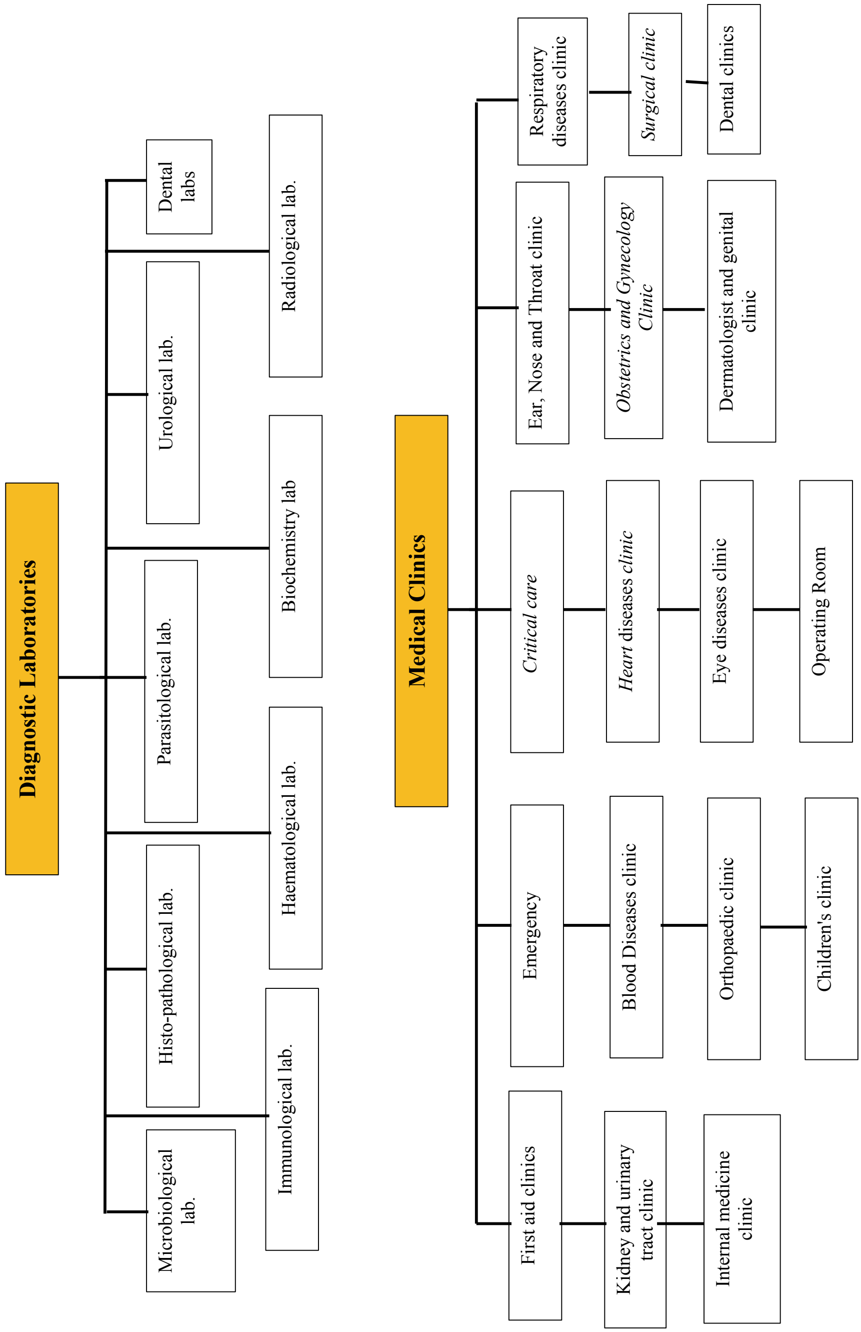

Fig. 10.4 Sources of clinical wastes

10.4 Current Legislation for Clinical Waste Management

Many of the developed countries in Europe regulated a legislation for the production, storage, transportation, treatment, and final disposal of the clinical wastes. The legislation for the clinical waste is different from that regulated for the infectious waste and healthcare waste. In terms of production, it has been mentioned that this process should be accomplished by an effective segregation at source which represents the key factor in the clinical waste management strategy as well as facilitate the storage transportation and the treatment process for the wastes. Moreover, the disposal of clinical wastes into the landfill is becoming non-acceptable discouraged, while the treatment process by the incineration has several disadvantages such as production of toxic by-products (Moritz 1995). The proper waste handling should be started with a proper segregation and resource recycling (Cheng et al. 2010; Efaq et al. 2015). The segregation process at the generation source aims to separate a large proportion of the wastes which are not classified within the infectious and hazardous or clinical waste and then co-disposing with the household waste to municipal incinerators or landfill sites. The application of segregation process would lead to minimize the economic cost for the clinical waste management which needs a special transportation and treatment due to their hazardous contents. The storage process for the clinical wastes before the treatment process or the transportation to the incineration is a temporary process; according to Saini et al. (2004), the storage period should be less than 24 h. However, in the small clinics, these wastes are collected and stored for few months (Noman et al. 2016b). The long period of the storage represents a contamination point for the hospital or clinics with several pathogens.

Some of the clinical wastes such as radioactive and pharmaceutical wastes are classified as special wastes which have specific regulation in UK for their management process. In USA, EPA is proposing to regulate hazardous pharmaceutical waste under the Universal Waste Rule, but there are no specific regulations that have been reported up to date.

Moreover, the legislation and regulation for the clinical waste management aim mainly to reduce the health risk of these wastes. These legislations are regulated in the UK (Department of Health), USA (Environmental Protection Agency), Australia (Ministry of Health), Turkey (Ministry of Environment and Forestry), and in Malaysia (Environmental Quality ACT 1974). In Taiwan, the clinical wastes are classified within the category of hazardous industrial wastes, which include the infectious materials generated from the healthcare facilities as well as the wastes with a toxic nature (TEPA 2004). The country has very strict regulations for clinical waste management. However, the treatment process is conducted by only the incineration. In the regulations related to the management of the clinical wastes, these wastes should be stored temporarily at the point of generation before it is transported and treated in a treatment facility such as incineration. Three-container systems have different colors which should be used for the clinical waste segregation, the infectious wastes are collected in a yellow/red bag, while the general wastes are collected in a black bag; moreover, the sharps are collected in specific container. These regulations are not applied among many of the developing countries such as Yemen, Iran, and Tanzania due to the absence of awareness about the health risk associated with the clinical wastes (Mato and Kaseva 1999; Efaq and Al-Gheethi 2015).

10.5 Conclusion

Healthcare wastes represent a serious risk for the community as it is heavily loaded with the infectious agents which may survive for long time in the environment. The best management might contribute effectively in minimizing the health risk and protect the environment. The management of the clinical wastes in terms of segregation, storage, transportation, and treatment as well as the disposal process would contribute effectively in the reduction of quantities and risk of clinical wastes.

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