Chapter 11
Recycle of Greywater for Microalgae Biomass Production

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Abstract The potential of greywater to be used as a production medium for biomass lie in the high concentrations of nitrogen and phosphorus as well as the organic matter necessary for microalgae growth. Microalgae have high potential to adapt and utilise nitrogen, phosphate and other nutrients available in wastewater. Other factors which affect the production of biomass in microalgae include light, temperature, aeration and mixing. The effect of pH might also contribute to the quality and quantity of the produced biomass. The critical step in the production of biomass lies in the harvesting of microalgae cells, extraction of the lipids, proteins and carbohydrates. The objective of this review was to identify the criteria required for selecting greywater as a production medium and microalgae species. The harvesting and extractions techniques used in this process are also discussed and also the quality of the produced biomass and the further utilisation based on the toxicity, nutrients values and microbiological aspects.

Keywords Greywater · Microalgae biomass · Quality · Harvesting process
Application

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11.1 Introduction

The improper management of the greywater is a major challenge in most of the developing countries. The discharge of these wastes into the environment constitutes many adverse effects on the natural biodiversity. Conversely, the characteristics of the greywater in terms of the nutrients and elements make these wastes a suitable production medium for the generation of biomass. Among several types of the microorganism which might be cultured in the greywater, the microalgae are the most appropriate organisms because they have chlorophyll which can obtain the required energy from the light by the process of photosynthesis. This process is used by the microalgae cell to convert light and CO_2 into glucose which is the main substrate in the anabolic pathways and production of biomass (Shekhawat et al. 2012).

The generation of microalgae biomasses and their application in the different sectors of the life have been started since the 1970s. However, the applications such as biofuel production and bio-generation of bio-products such as a source of valuable chemicals, food additives and pharmaceuticals have increased significantly since 2008 (Pahazri et al. 2016). In the recent years, several companies are working on producing microalgae biomass in the marine and freshwater (Jais et al. 2017). The most common microalgae species used are *Botryococcus sudeticus*, *Dunaliella* sp., *Chlorella vulgaris*, *Haematococcus pluvialis*, *Nannochloropsis oculata* and *Spirulina platensis*. It has been estimated that the total amount of the *Haematococcus* sp. by 30 tonnes/years and that for *Spirulina* sp. by 20 tonnes/year. It is estimated that more than 6000 l of water are required to produce biomass yield enough to generate one litre of algal oil based on the conventional systems of cultivation, which indicates that the use of a large-scale algal cultivation in freshwater is not an economically suitable option due to the problems of water shortage in many of the developing countries (Ozkan et al. 2012). In this review, the wastewater might provide the alternative source of the water.

Many of the developing countries in the East Asia and Middle East countries have the environmental conditions suitable for the production of microalgae biomass. The microalgae biomass generated from the cultivation of microalgae species in the greywater have several applications in the industry, agriculture and medical activities. The commercial bio-products generated from the microalgae biomass are cosmetics, organic fish feed, human nutrition, pharmaceutical products and animal feed as well as biodiesel (Bala et al. 2016; Jais et al. 2017). However, there are several challenges which should be considered to ensure the recycling of the greywater as a production medium for microalgal biomass. The operating and harvesting process of biomass yield, as well as extraction of protein and lipids from the biomass, are the main points which are discussed in this chapter.

11.2 Recycle of Greywater for Microalgae Biomass Production

The recycling of greywater is consistent with the concept of zero discharge raised in 1980 for industrial wastewater and aims to recycle or reuse wastes (Efaq et al. 2015). The utilisation of effluents as a media for the production of enzymes such as β-lactamase and cellulase by bacteria has been investigated by Al-Gheethi (2015) and Al-Gheethi and Norli (2014). Many of the wastewater including municipal and industrial wastewater as well as dairy industry has been used as a culture medium for the microalgae biomass. Microalgae have high contents of carbohydrates, lipids and proteins. Therefore, it represents a good nutrition source as an animal feeds.

The recycling of greywater as a production medium for the microalgae biomass relied on the chemical, physical and biological characteristics of the greywater. The nutrients represent the key factors for the microalgae growth. The greywater has a content of nitrogen, phosphorus and trace elements in the range required for microalgae growth and production of biomass yield (Pahazri et al. 2016). Others parameters such as pH, temperature and light are also important, but these parameters are adjustable. The microbiological aspects of the greywater should be more investigated. Some of the microalgae could grow well in the greywater since it has the potential to compete with the indigenous organism where other microalgae have failed to grow. The relationship between microalgae and bacteria in the greywater has been investigated (Al-Gheethi et al. 2017). The secondary metabolic of the bacterial cells such as the release of CO_2 might induce the microalgae growth. Some of the microalgae have antibacterial activities which can inhibit the bacterial growth. In contrast, some of the bacterial species have algicidal activities. Therefore, the selection of microalgae species represents the bottleneck in the recycling of greywater as a culture medium. The other point is the presence of the pathogenic bacteria which might survive during the production process of the microalgae biomass, and they are harvested with the biomass yield and thus limit the application of biomass yields. One of the solutions to overcome this problem is to sterilise greywater before the recycling.

The choice of microalgae species is an important parameter to be considered, the ability of microalgae to survive under hard environmental conditions reflect its potential to grow in the greywater and thus results to overproduction of biomass yields. In this case, the best option to consider is to use indigenous microalgae species isolated from the surrounding environment. It is estimated that the microalgae species are more than 200,000 types; many of these species have the ability to survive in extreme conditions (Kalin et al. 2005). The ability of the microalgae to survive in different environmental conditions is attributed to their rapid rate of acclimatisation to the surrounding environment even with low concentrations of the nutrients. This process is called the natural selection process. However, the selected microalgae species in the biomass production should be non-pathogenic and should not have the potentials to produce toxins, since these toxins in the biomass yield might limit the application of biomass as fish or animal feeds or as soil fertilisers.

Microalgae are known to be autotrophic organisms; however, for overgrowth they require some of the nutrients in terms of nitrogen and phosphate as well as Fe, Mg, Na and Cu, as trace elements (Rahman et al. 2012). Therefore, the greywater might provide these requirements for the microalgae better than the freshwater. *Chlorella* sp., *Botryococcus* sp., *Euglena* sp. and *Scenedesmus* sp. are among the different species of the microalgae which have been investigated for their growth in the wastewater and exhibit good biomass production (Godos et al. 2012). The nutrients and trace elements in the greywater are the main factors which determine the potential of the greywater to act as a production medium for microalgae growth. Many of the other factors should be adjusted to improve the high quality and quantities of the biomass yield. In a view to examine the factors affecting microalgae growth and the characteristics of the greywater, it can be noted that pH, temperature, light, aeration, CO_2, salinity and mixing are the factors which need to be considered. These factors play a secondary effect on the microalgae growth but also contribute significantly to the amount of produced biomass (Abdel-Raouf et al. 2012).

11.2.1 Factors Affecting the Biomass Production in Greywater

The factors affecting the production of microalgae biomass are not independent variables as they interact together. The concentration of pH of the greywater ranges from 6 to 8, this range is within the optimal pH for microalgae growth which is between pH 7.5 and 11 for most of the microalgae species such as *Scenedesmus* sp. and *Chlorella vulgaris* (Sengar et al. 2011; Gong et al. 2014; Jais et al. 2017). The pH contributes to the microalgae growth which has direct effect or influence on the diffusion and transportation process of the nutrients through the cell membrane by the assimilation process of nutrients by microalgae cells as well as the activity of chlorophyll. The pH for CO_2 capturing from the atmosphere by the microalgae cells ranges from 7 to 9.5. However, pH during the growth of microalgae is changed. These changes depend on the photosynthesis activities, microalgae initial inoculums, aeration as well as the nature of nitrogen source. For instance, pH increases as a result of the photosynthetic CO_2 assimilation; this process takes place faster in the heavy inoculums and provides the high concentration of CO_2, HCO_3^- and H_2CO_3, while in the role to keep the pH within the optimal range for algal cultivation (Yaakob et al. 2014). In terms of CO_2, it should be noted that the maximum biomass of *Scenedesmus* sp. (0.2 g L^{-1}) and *Chlorella* sp. (0.12 g L^{-1}) was recorded with 24% of CO_2 concentrations (Makareviciene et al. 2014).

In contrast, pH is decreased when the concentration of ammonia is used as nitrogen source due to the release of H^+ ions which reduces the pH value below 4 (Sengar et al. 2011). In this case, the pH of the culture should be adjusted by using buffer solutions such as K_2HPO_4 to keep the pH value constant during the biomass production. Nevertheless, the authors have decided against the application of chemical additives into wastewater used as a production medium, since it might impact on the quality of biomass yield. Therefore, this problem can be overcome by using the continuous culturing system in which a balance between input and output greywater is adjusted (Brar et al. 2017). In contrast, the presence of nitrate as a secondary nitrogen source in the greywater medium contributes to the increase of pH value (Arumugam et al. 2013). However, in some of the microalgae species such as *Scenedesmus bijugatus*, nitrate is the preferred nitrogen source for the growth and production of biomass in the wastewater. The nitrogen contents in the microalgae cells range from 25 to 40% (Riano et al. 2016). Some of the microalgae species have the ability to fix nitrogen from the atmosphere but this pathway might be used by the microalgae cells as the alternative source if the concentration of nitrogen sources in the greywater is insufficient. The nitrogen contributes more than 4% in the production of microalgae biomass and approximately 10% in the production of lipids. However, it has been reported that some of the microalgae species such as *Neochloris* sp., *Tetraselmis* sp., *Nannochloropsis* sp. and *Scenedesmus* sp. produce maximum lipids and carbohydrate in the medium with low concentrations of nitrogen substances (Minhas et al. 2016).

Phosphorus concentration in the greywater is one the main factors which might induce or inhibit the microalgae growth. The microalgae need these elements between 0.03 and 0.06%, but it is necessary for the cell metabolism, since the phosphorus plays important elements for saving of the energy as adenosine triphosphate ATP required for the metabolic and anabolic pathways (Yaakob et al. 2014). Orthophosphate (PO_4^{3-}) is the superior form of most of the algae; the microalgae cells store the phosphorus in the form of polyphosphate to face the deficiency during the growth (Rasala and Mayfield 2015). As mentioned above, the interaction between the parameters in the greywater occurs in the case of phosphorus which is easy binds to Fe ions. Therefore, in some case, the concentrations of Fe ions in the greywater should be reduced to provide a favourable medium for biomass production (Yaakob et al. 2014).

Light is the sole source of energy in the microalgae; therefore, it represents the backbone for the production of microalgae biomass in the greywater. Even in the presence of high concentrations of the nutrients, the absence of the light makes the greywater not useful for microalgae growth. The limitation for the penetration of light through the greywater is that the turbidity, low density of the sunlight required for the microalgae growth which. For this reasons, the turbidity should be reduced before the recycling of the greywater as a culture medium. Meanwhile, the high density of the light might affect negatively the microalgae growth and the optimum light density recommended for high microalgae growth which is estimated to be 600 ft. candles (McKinney 2004). The optimum light density for *Spirulina platensis* was 232.26 fc, while it was 225 fc for *Chlorella kessleri* and 0.657–1.34 fc for *Botryococcus braunii* (Lee and Lee 2001; Qin and Li 2006; Fagiri et al. 2013). Moreover, the microalgae have developed the potentials using special mechanism to survive in the high density of the light; this takes place by the moving deeper or by releasing internal gases which are allowed to sink to desired level. In contrast, in the weak light, the microalgae are grown on the surface of the waters. Therefore, to understand the microalgae behaviour for the light might improve the overproduction of the biomass, since one of the problems which was recorded in the production of *Botryococcus* sp. in the greywater incubated under the direct sunlight was the participation of the microalgae cells. These observations were noted for several studies conducted in Malaysia which have 10,000 fc of the sunlight intensity. The effect of the sunlight on the microalgae growth also depends on the type of the greywater. The laboratory experiments found that *Botryococcus* sp. failed to grow in some greywater samples while grown more on the others. But in the view of greywater characterises, the inhibition of the microalgae growth might be due to the presence of xenobiotics organic compounds (XOCs). The toxicity of these is discussed more in Chap. 5.

The photoperiod of the microalgae growth is in the range of 12/12 to 18/6 h of light/dark (Mahale and Chaugule 2013). This cycle period is due to the nature or mechanism of the photosynthesis which is carried out in light and dark stages. In the light stage, the light is converted in the chloroplast into energy and then to $NADPH_2$ and ATP which are used to synthesise lipids, starch and sugar while in the dark cycle, the stored energy is used for the metabolic and anabolic reactions to synthesise amino acids (Jacob-lopes et al. 2009; Pérez-Pazos and Fernández-Izquierdo 2011). Therefore, the microalgae also exhibit a detectable growth in the dark. From the studies reported in the literature, the period of 12/12 h of light/dark is not standard for all the microalgae species. In some cases, the increase of the light periods is associated with maximum biomass yield and lipid contents, where the long period of the light cycle might improve the quality and quantity of the microalgae biomass by 100% (Rai et al. 2015). It is worthy of note that the light might induce the production of some of the specific compounds as in the case of *S. platensis* which requires 3500 lx for the production of carotenoid which requires only 2000 lx to achieve high biomass production (Kumar et al. 2015). There should be a clear understanding of the differences between the biomass yield and production of bio-products. The biomass production is a process that takes place during the log phase of the microalgae growth in which the cells used up the energy in the anabolic pathways and build more amino acids and thus increasing the microalgae cells which imply that more biomass is produced. In contrast, the production of specific compounds is a process that takes place during the stationary phase in which the cells have stopped the reproductions and the mature cells have high metabolic activity and thus more secondary metabolic bio-products are generated. Therefore, the cultivation of microalgae is proposed to be carried out in two stages. The first stage should be conducted with 12/12 light/dark to allow for the microalgae cells to grow and multiply and then produce more biomass. In the second stage, the period of lighting should be increased to induce the microalgae for producing the specific compounds.

The availability of trace elements in the greywater plays the very remarkable role in improving the microalgae growth. The elements such Ca, K, Mg, Ni, Cu and Mn act as cofactors for the many metabolic enzymes in the microalgae cell (Jais et al. 2017). For instance, Fe ions act as the electron, and Mg is required for the chlorophyll, while others such as Ca, K, Zn, Cu and Mn are required by algae to sustain the living cells. The greywater is rich with these elements which resulted from the detergents and others chemical compounds. The heavy metals might also be present in the greywater, but the reports indicated that they occur with a concentration below the sublethal levels (Wurochekke et al. 2016).

Temperature is a critical factor which has a real role in enhancing or inhibiting the microalgae growth. This role belongs to their effect on the diffusion and transportation of nutrients, protein and chlorophyll contents, metabolic activities, respiration intensity enzymatic reaction, specific affinity for nitrogen and phosphorus, CO_2 fixation as well as the cellular chemical composition and growth rate (Xin et al. 2011; Jais et al. 2017). Unlike the other factors which the highest effect might lead to inhibit or inactivate the microalgae cells, the high temperature might kill the cells by destroying the enzyme structure and function. Most of the microalgae grow at the ambient temperature (15 and 25 °C), some of them such as *C. vulgaris* have a maximum temperature growth reach of 30 and 35 °C, and *Spirulina* sp. has temperature growth range between 20 and 40 °C, while the temperature growth range of *Scenedesmus* sp. is between 10 and 40 °C (Cassidy 2011). Moreover, both light and temperature changes might be overcome by using the indigenous microalgae strains obtained from the surrounded and local environments.

The microalgae species which have been reported to produce biomass yield in different wastewater samples are listed in Table 11.1.

Based on the aforementioned, it can be concluded that the interactions between the factors affecting the microalgae growth and biomass production need to be optimised to detect the best operating parameters required for overproduction of microalgae. One of the best software programs to study the optimisation process is the response surface methodology which has been used by several authors to optimise the biotechnology applications including the biomass production (Efaq et al. 2016; Adeleke et al 2017; Hauwa et al. 2017b).

11.3 Harvesting Techniques of Microalgae Biomasses from the Culturing Media

The qualities and quantities of the biomass yield in the greywater depend mainly on the harvesting methods efficiency and recovery percentage from these wastes. Table 11.2 presents the percentage efficiency of the microalgae biomass using different harvesting techniques.

The harvesting microalgae biomass might be performed by physical, chemical and biological techniques or a hybrid system between them. The mechanical methods are superior due to the possible recycling of the culture media of the microalgae, since no chemical substances have been added, but the selection of the favourable method is based on the efficiency of the method with respect to other methods. The considerations for choosing the appropriate method depend on several factors which are related to the final utilisation and the economic and commercial points, since it is estimated that the harvesting process cost represents 20–30% of the total production cost (Pahazri et al. 2016). The factors affecting the harvesting processes include types

Table 11.1 Microalgae species grown in various types of wastewater

Microalgae	Type of wastewater	References
Botryococcus braunii	Greywater	Gokulan et al. (2013)
B. braunii	Household greywater	Gani et al. (2015)
Botryococcus sp.	Swine wastewater	Liu et al. (2013)
C. minutissimum	Sewage wastewater	Azarpira et al. (2014)
C. pyrenoidosa	Textile wastewater	Pathak et al. (2014)
C. sorokiniana Desmodesmus communis	Wastewater	Yao et al. (2015)
C. vulgaris	Chemical manufacturing wastewater	Rao et al. (2011)
C. vulgaris	Rubber latex concentrate processing wastewater	Bich et al. (1999)
Chlorella sp.	Poultry wastewater	Agwa and Abu (2014)
Chlorella sp.	Centrate Municipal wastewater	Min et al. (2011)
Chlorella saccharophila, *Chlamydomonas pseudococcum*, *Scenedesmus* sp., *Neochloris oleoabundans*	Dairy farm wastewater	Hena et al. (2015)
Chlorella vulgaris	Wastewater	Sengar et al. (2011)
- *Gloeocapsa gelatinosa* - *Euglena viridis* - *Synedra affinis*	Drain water	
Nostoc sp.	Dairy effluent	Kotteswari et al. (2012)
Pithophora sp.	Dairy wastewater	Silambarasan et al. (2012)
Phormidium sp.	Cattles laughter house wastewater	Maroneze et al. (2014)
S. obliquus *C. sorokiniana*	Raw sewage	Gupta et al. (2016)
Scenedesmus dimorphus	Anaerobically digested palm oil mill effluent	Kamarudin et al. (2013)
Scenedesmus sp.	Swine wastewater	Kim et al. (2007)
	Aqueous Solution	Xin et al. (2010)
	Swine wastewater	Michel et al. (2016)
	Municipal wastewater	Alva et al. (2013)
	Artificial wastewater	Song et al. (2014)
	Secondary wastewater	Kim et al. (2015)
	Sewage wastewater	Lekshmi et al. (2015)
	Tannery wastewater	Ajayan et al. (2015)
Spirulina platensis	Sago starchy wastewater	Phang et al. (2000)

Table 11.2 Harvesting methods of microalgae by different techniques

Algae species	Harvesting method	Efficiency percentage (%)	Reference
S. quadricauda	Flotation methods (SDS + Chitosan)	95	Chen et al. (1998)
Chlorella sp.	Flotation method (SDS + Chitosan)	85–90	Liu et al. (1999)
Scenedesmus quadricauda	Ultrafiltration	92	Zhang et al. (2010)
Parachlorella spp., *Scenedesmus* spp., *Phaeodactylum* spp., *Nannochloropsis* spp.	Cationic starch	90	Vandamme et al. (2010)
C. sorokiniana, *Scenedesmus obliquus*, *Chlorococcum* sp.	$FeCl_3$ and $Fe_2(SO_4)_3$	66–98	De Godos et al. (2011)
Chlorella sp.	Sedimentation and filtration	90.8	Li et al. (2011)
Chlorella sp.	Chitosan	99	Ahmad et al. (2011)
Chlorella vulgaris	Magnetic filtration	90	Ruiz-Martinez et al (2012)
Chlorella vulgaris	Submerged microfiltration	98	Bilad et al. (2012)
Chlorella vulgaris	*Maringa oleifera*, *Aluminium sulphate*	85	Teixeira et al. (2012)
Dunaliella salina	Aluminium sulphate	95	Hanotu et al. (2012)
	Ferric sulphate	98	
	Ferric Chloride	98.7	
Chlorella vulgaris	Chitosan	99	Rashid et al. (2013)
Nannochloris sp.	Centrifugation	>90	Dassey and Theegala (2013)
S. obliquus	Filtration	99	Ji et al. (2013)
Nannochloropsis oculata	$FeCl_3$ and $Fe_2(SO_4)_3$	93.80	Surendhiran and Vijay (2013)
		87.33	
Chlorella sp., *Chlamydomonas* sp.	Cationic guar gum (CGG)	94.5	Banerjee et al. (2013)
		92.15	
C. vulgaris, *S. obliquus*	Saponin and chitosan	93	Kurniawati et al. (2014)
C. protothecoides	Cationic starch	84–90	Letelier-Gordo et al. (2014)
Chlorella sp.	*Moringa oleifera*	90	Hamid et al. (2014)
S. dimorphus, *S. minutum*	Centrifugation	96	Gentili (2014)
		91	
Botryococcus sp.	*Maringa oleifera*	90	Hauwa et al. (2017a)

of culture media as wastewater or freshwater as well as the size of the microalgae cells (Barros et al. 2015). Microalgae cells grow in the culture medium in two layers, the surface layer (biofilm) which represents 2–7% of the total microalgae biomass and can be harvested either by the flotation or coagulant additives to enhance the participation process. In contrast, more than 90% of the biomass yield is suspended in the production medium and this quantity needs a critical selection for a suitable method for achieving the maximum recovering percentage (Atiku et al. 2016). In this section, the harvesting methods used for recovering of microalgae biomass from different culturing medium are discussed.

11.3.1 Centrifugation

The centrifugation method is the suitable methods used for recovering the microalgae biomass with small sizes. Centrifugation exhibit is effective and fast for harvesting the biomass without the need for chemical additives, it can achieve 80–95 of the recovery percentage within 2–5 min at 13,000 × g for *Tetraselmis* sp. and *Chaetoceros calcitrans* and 30% at 6000 × g (Dassey and Theegala 2013). The absence of chemical additive might assist in the storage of the biomass for a long time without any negative effect on the quality. Moreover, the process supposed to be carried out at appropriate speeds to avoid the damage of the cells by the high gravitational and shear forces as in the cases recorded for *Tahitian Isochrysis*, *Chaetoceros muelleri* and *Pavlova lutheri*, which are totally restricted due to the absence of hard cell walls (Caixeta et al. 2002; Knuckey et al. 2006). Nevertheless, the destruction of the microalgae cells might be an advantage for the centrifugation process, since no more extraction process is required for the biomass, but this case is good if the microalgae biomass yield will be used for biodiesel production. Among different designs of the centrifuge, the tubular bowl centrifugation is the more suitable for the small laboratory scale (Yaakob et al. 2014). Other limitation of the method includes the cost and increase in the temperature during the centrifugation. The temperature might be adjusted by providing the centrifugation with ice bath, but the cost is still the main challenge.

11.3.2 Membrane Filtration

The filtration techniques are the best alternative way in order to avoid the destruction of the microalgae cells as a result of high speed on the centrifugation (Atiku et al. 2016). However, this technique is more appropriate for the microalgae biomass with large sizes such as *Arthrospira* sp. but it is also dependent on the pore size of the used filter (Park et al. 2011). One of the challenges for using the membrane filtration is due to the microbial and microalgae growth on the surface of the membrane filter which leads to reduction in the effectiveness of this process in achieving the high recovering percentage of biomass from the culture medium as well as contamination of the harvested biomass (Bohdziewicz et al. 2003; Barros et al., 2015). Tangential flow filtration is a promising technique for the recovery from microalgae cells from the culture media with the efficiency ranging from 70 to 89% without any changes in the cell morphology or structure (Petrusevski et al. 1995). Micro-strainers is another type of the harvesting process which is dependent on the filtration mechanism; this process has simple implement and operation as well as can be used in both directions; therefore, no microalgae growth is accumulated on the surface (Chen et al. 2011).

11.3.3 Sedimentation

Very few studies have been reported on the use of sedimentation method for harvesting of the microalgae biomass. This process takes place in nature as a response to the changes in the pH of water, which leads to the settlement of the microalgae cells as a function of the gravity without the need for chemical additives. The auto-flocculation and bio-flocculation are one of the natural sedimentation methods which take place due to the extracellular polymeric substances (EPS) produced by some microalgae species such as *Scenedesmus obliquus*, *Micractinium* sp. and *Chlorella* sp. or by bacteria such as *Solibacillus silvestris*. In this case, the microalgae cell are agglomerated and then precipitated by the sedimentation (Guo et al. 2012; Wan et al. 2013; Ndikubwimana et al. 2014). The efficiency of this method is acceptable and inexpensive for the microalgae species with large cell size such as *Arthrospira* sp. and *Nannochloropsis* sp. In some cases, it can be accelerated by coagulants additives which might achieve 99% of the recovery (Barros et al. 2015).

11.3.4 Flotation

The flotation method contributes effectively to the harvesting of microalgae with cells size ranging from 10 to 500 μm (Hanotu et al. 2012). The air injection system produces air bubbles between 700 and 1500 lm and enhances in the process the efficiency of the recovery percentage of the biomass from the culture media (Rubio et al. 2002). In the dispersed air flotation (DAF), the air bubbles are between 10 and 100 lm, which make it more efficient than the settling process (Uduman et al. 2010). The combination between DAF and electro-flocculation method has achieved 98.9% of the recovery percentage of *Botryococcus braunii* within 14 min (Xu et al. 2010). The modification of the hydrophobicity of bubble surfaces by algogenic organic matter (AOM) or with the surfactant cetyltrimethylammonium bromide (CTAB) recorded high improvements for the recovering methods (Cheng et al. 2011). DAF has one limitation which lies in the oversized bubbles that could lead to the breakup of the flocs (Pragya et al. 2013). Flotation technique is used extensively in wastewater treatment processes after the removing of suspended solids by the coagulation process (Sim et al. 1988). It is more suitable for recovering of *Anabaena* sp., *Microcystis* sp. and *Arthrospira* sp., which have gas vesicles and thus have low density and are not harvested by centrifugation. Dispersed air flotation (DiAF) is similar to DAF, but the bubbles are generated by passing air continuously through a porous material. This process needs less energy. However, it needs more expensive equipment for the generation of the air bubble. The combination between DiAF and bio-surfactant saponin enhanced the recovery percentage of *S. obliquus* and *C. vulgaris* to more than 90% (Kurniawati et al. 2014). In ozonation-spread flotation (ODF), the recovering of microalgae biomass take place as a function of the interaction between negative functional groups on the surface of microalgae cell and positively charged bubbles. The method contributed in the extraction of lipids by 24% from *C. vulgaris* (Barros et al. 2015). However, the concern in the use of ozone is the formation of secondary products which might have carcinogenic effects (Rawat et al. 2011).

11.3.5 Chemical Flocculation and Coagulation

The flocculation and coagulation as a function of chemical coagulants are the most common technology used for the recovering of microalgae biomass from the culturing system. The coagulants used include polymers (organic and inorganic) or chelators (metal salts and alum) (Atiku et al. 2016). The polymers are more efficient than metal salts for many of microalgae species such as *C. vulgaris*, *Muriellopsis* sp., *Scenedesmus subspicatus*, *Chaperina fusca* and *Scenedesmus* sp. (De Godos et al. 2011). The hybrid flocculation system consists of metal salts and polymers might achieve more than 90% of the *C. vulgaris* (Gorin et al. 2015). The mechanism in which the flocculation by the multivalent metal ions acts to the harvest of microalgae biomass is explained based on the negative charge of the functional groups on the microalgae cell wall and the hydrolysed metals with positive charges. This process acts mainly as a function of pH where the optimal pH is 7, at which the flocculation achieves high efficiency, the functional group interacted with the ions to become unstable and then aggregated to form the flocs. The efficiency of flocculation to achieve high recovery percentage is a response for the type of coagulant (dosage of the coagulant, charge density and molecular weight), microalgae cell concentrations, operating parameters (pH, mixing speed, retention time and temperature (Hauwa et al. 2017a).

The concerns related to the use of flocculation method with the chemical coagulants are the health risks associated with the toxic by-products. The alum and acrylamide have been recorded by the authors as disease-causing agents such as Alzheimer's as well as carcinogenic substances (Ahmad et al. 2011). The concerns should be considered in the further application of harvested biomass as animal or fish feeds or fertilisers (Hamid et al. 2014). In this case, the alternative compounds which have been suggested by the authors are the polyelectrolytes compounds as well as the biodegradable organic polymers such as starch and chitosan. Both starch and chitosan recorded increasing efficiency (80–96–99%) of the microalgae biomass from the culture media, respectively (Vandamme et al. 2010; Rashid et al. 2013). Porcelanite is another alternative coagulant for the recovering of microalgae biomass, it has not been investigated before, but the chemical composition of porcelanite in terms of presence Al_2O_3, Fe_2O_3, CaO, MgO, CaO and TiO_2 indicated that it will have high harvesting efficiency (Atiku et al. 2016).

11.3.6 Natural Flocculation and Coagulation

The new directions in the field of harvesting method for microalgae biomass from the culturing process are by using the natural coagulants, which exhibited higher effectiveness in comparison with the chemical coagulants. One of the best advantages of using natural coagulants is the absence of chemical additives; rather than this, the natural coagulants might have more protein substance which might improve the quality of microalgae biomass. Among different natural coagulations, *Moringa oleifera* and *Strychnos potatorum* are the most studied; *M. oleifera* has recorded between 85 and 99% of the *Chlorella* sp. and *Botryococcus* sp. recovery from different culture media (Hamid et al. 2014; Hauwa et al. 2017a). The mechanism which makes the natural coagulants to have high efficiency for the harvesting of microalgae biomass is the presence of active polyelectrolytes with positive charges as well as their potential to dissolve in the water (Imtiazuddin et al. 2012). *M. oleifera* as a natural coagulant is non-toxic and has high efficiency with the low dosage, as well as lipid and protein contents with specific functional groups which make it one of the best alternative and natural coagulants for the chemical substances (Hauwa et al. 2017a). *S. potatorum* has also similar composition with *M. oleifera* such as carbohydrates, lipids and alkaloids so it plays an important role in the recovering of the microalgae biomass from the production media (Pahazri et al. 2016). The use of γ-glutamic acid in the harvesting of *N. oculata*, *Chlorella protothecoides*, *C. vulgaris*, *Phaeodactylum tricornutum* and *B. braunii* has recorded 90% of the harvesting efficiency (Zheng et al. 2012). Ecotan and Tanfloc coagulants have achieved 90% of biomass recovery from the culture medium (Gutiérrez et al. 2015), while cationic guar gum (CGG) recovered 94.5% of *Chlorella* sp. and 92.15% of *Chlamydomonas* sp. (Banerjee et al. 2013). In contrast, the use of nanotechnology in the preparation of coagulants such as cellulose nanocrystals (CNCs) increased the efficiency of the harvesting of microalgae biomass to 100% (Vandamme et al. 2015). The natural coagulants are biodegradable substances which are not toxic to human and biodiversity in nature.

11.3.7 Advance Technologies for Harvesting of Microalgae Biomass

The electrocoagulation is one of the most promising technologies for the harvesting of the microalgae biomass and is used as an alternative technology for the traditional methods which have several limitations (Matos et al. 2013). This technology is also used for the removal of xenobiotic organic compounds (XOCs). It depends on the release of metal ions by electrolytic oxidation of the anode which reacted with the microalgae cells to form the flocs. The flocs are separated by the sedimentation with 97% of the recovery percentage for some of the microalgae species such as *Nannochloropsis* sp. (Uduman et al. 2011).

The harvesting of the microalgae biomass from the culture medium by the magnetic separation acts based on the functionalized magnetic particles such as cationic polyelectrolytes (Fe_3O_4) and an external magnetic field with negative charges such as microalgae cells (Toh et al. 2012). This technology has achieved 95% of the recovery of *Chlorella ellipsoidea* (Hu et al. 2014). The magnetic particles are adsorbed on the functional groups of the microalgae cell wall by electrostatic bonds (Lim et al. 2012). Many of the magnetic particles have been used for recovery of microalgae from the production medium; the silica-coated magnetic particles exhibited more than 95% of the recovery of *Chlamydomonas reinhardtii*, *C. vulgaris*, *P. tricornutum* and *Nannochloropsis salina* (Cerff et al. 2012). Moreover, the developments in the magnetic separation have included the use of nanotechnology for the preparation of Fe_3O_4 nanoparticles, which exhibited more than 98% of *B. braunii*, *Corymbia ellipsoidea* and *Nannochloropsis maritima* (Xu et al. 2011). The low-gradient magnetophoretic separation coupled with iron oxide nanoparticles (NPs) and functionalized with cationic polyelectrolyte (diallyldimethylammonium chloride) (PDDA) harvested *Chlorella* sp. by 99% (Lim et al. 2012), while 95% of *C. vulgaris* recovery was achieved with iron oxide magnetic microparticles (IOMMs) (Prochazkova et al. 2013).

The milking technology is common for the harvesting of microalgae biomass as live cells from the low productive medium used for the extraction of high-value compounds such as carotenoids from *Arthrospira platensis* (Liu et al. 2009). In the milking process, the microalgal biomass is not harvested, rather than it performed like a continues culture system, where new cells are generated and grown in the log phase while others are in the stationary phase which is induced by the addition of the chemical substance to produce specific compounds. The addition of dodecane into the bioreactor of microalgae growth has improved the production of β-carotene (Hejazi et al. 2004).

11.4 Drying and Applications of Microalgae Biomass

In order to facilitate the management of the harvested microalgae biomass, it supposed to dehydrate the water contents and reduce the level of the moisture by the drying methods. However, the selection of the drying methods should be considered based on the future utilisation of this biomass. The drying process should have no effect on the quality of the biomass and the nutrients value as well as the chemical composition. The chemical composition of different microalgae biomass is presented in Table 11.3.

In some cases whereby the microalgae biomass is stored before the final utilisation, the conditions of the storage system are expected to have no negative effect on the biomass. The considerations should also include the selection of extraction method for lipids and protein from the biomass. The main challenge of the drying, stored and final utilisation is the destruction in the chemical composition and loss of the nutrients values of the biomass as well as the contamination by the bacteria or fungi which might grow on this biomass due to the high contents of the organic matter (Jais et al. 2017). Therefore, the dying methods need to have more than one function such as dehydration of water and disinfection of the biomass. Among several methods of the drying methods, the solar radiation might be the best promising technique due to their ability to remove the water and inactivates the pathogens as well as the degradation of some of the micro-pollutant organic compounds from the greywater during the harvesting process (Al-Gheethi et al. 2013; Atiku et al. 2016). However, the solar radiation is more applicable in the arid and semi-arid countries (Al-Gheethi et al. 2015).

Table 11.3 Protein and lipid contents in the microalgae biomass generated in different types of wastewater

Type of wastewater	Microalgae species	Protein content (%)	Lipid content (%)	References
Olive oil mill wastewater	*Spirulina platensis*	38.13	16.91	Wang et al. (2010)
Meat processing wastewater	*Chlorella* sp.	68.65	17.54	Lu et al. (2015)
Piggery wastewater		NA	21	Kuo et al. (2015)
Piggery wastewater	*Scenedesmus* sp.	NA	31	Hamid et al. (2014)
Dairy farm wastewater		45.09	21.82	Michels et al. (2014)
Aquaculture wastewater	*Spirulina plantesis*	48.5	4.7	Guerrero-Cabrera et al. (2014)
Secondary effluents	*Chlorella sorokiniana*	22.36 mg L^{-1}	24.91 mg L^{-1}	Ramsundar et al. (2017)

In contrast, the freeze-drying method might be recognised as an alternative method for drying of biomass; this process is not very common but it has high potential to remove water without destruction of nutrients values in the biomass. However, the technique would not deactivate the microbial contents; therefore, it might be more suitable for the biomass used in the biodiesel production (Munir et al. 2013).

The final utilisation of the microalgae biomass might include whole algal products or compounds extracted from this biomass. Unlike the microalgae biomass harvested from the water medium, there many of aspects should be conserved as well in the biomass recovered from the greywater due to the complex structure of these wastes. In a view of the chemical and biological composition of the greywater, it can be found that there are many of the available pollutants such as heavy metals, XOCs and pathogens. These parameters should be removed if the biomass will be used as dietary supplements for humans and animals (Kang et al. 2013). Microalgae have many of the bio-products such as carotenoids, pigments, vitamin, polyunsaturated fatty acids (PUFAs) and antioxidants which of course have more advantages in comparison with those synthesised in the laboratory (Maizatul et al. 2017).

Another aspect which needs to be considered is the microbial contents which might also be harvested with the microalgae biomass. However, the microbial contents might have no more concerns when the biomass is subjected to the extraction methods since some of the extraction processes such as supercritical carbon dioxide (SC-CO_2) have dual role to extract the compounds from the biomass and to inactivate the pathogens (Efaq et al. 2017).

11.5 Conclusion

The potential of greywater for recycling as a production medium for microalgae biomass depends mainly on the purpose and final utilisation of the biomass. Nevertheless, the chemical composition of the greywater in terms of nutrients and growth factors available in these wastes makes it an alternative source for the waters. However, there are many of the considerations which need to be evaluated as well to recycle the greywater as a production medium. On the other hand, the harvesting methods represent the bottleneck in the production of microalgae biomass which might increase or decrease the quality of the biomass. Among most of the harvesting techniques, the advanced technology such as milking methods appeared to be the best option in terms of high quality of the extracted compounds and regeneration of the biomass for several times.

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