

# Phycoremediation of wastewaters: a synergistic approach using microalgae for bioremediation and biomass generation

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**Abstract** Discharge of untreated domestic and industrial wastewater into aquatic bodies is posing a serious eutrophication threat, leading to a slow degradation of the water resources. A number of physical, chemical and biological methods have been developed for the treatment of wastewaters; among these, the use of microalgae is considered as a more eco-friendly and economical approaches. Microalgae are versatile organisms which perform multiple roles in the environment—bioremediation of wastewater, gleaning of excess nutrients and in turn, generate valuable biomass which finds applications in the food, biofuel and pharmaceutical industries. They are currently being utilized to reduce the high nutrient load (especially N and P) from wastewaters, which fulfill the growth requirements of microalgae, making it a suitable cultivation medium for biomass production. The present review represents a comprehensive compilation of reports on microalgal diversity of wastewaters, followed by a critical overview of their utilization, suitability and potential in bioremediation vis-a-vis biomass production. This review also emphasizes the superiority of polyalgal and consortial approaches in wastewater treatment, as compared to the use of unialgal inocula, besides providing useful pointers for future research needs in this area.

**Keywords** Wastewater · Eutrophication · Microalgal diversity · Consortia · Nutrient removal · Biomass production

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## Introduction

Issues related to environmental pollution are becoming more serious with the increasing population, urbanization, industrialization and their indirect effects on ecosystem services (Rawat et al. 2011; Sood et al. 2012). The consequences include excessive generation of wastes/wastewater, release of untreated water into the freshwater resources and global warming, which are posing serious challenges for the scientific community, in terms of sustainability of our planet for the present as well as future generations. Each facet of environment pollution has its own list of problems which require specified know-how and technologies to meet and overcome the challenge. In this context, mixing of untreated wastewater in aquatic bodies is emerging up as one of the major issues that is challenging the stability of nations (Renuka et al. 2014; Yang et al. 2008). This is mainly due to the reason that the majority of populations in developing countries are directly or indirectly dependent on the freshwater resources for their day-to-day activities.

In developing countries like India, water scarcity is presenting serious issues, because of population explosion resulting in large quantities of sewage wastewater. Coupled with this, increasing industrialization, indiscriminate and excessive usage of fertilizers and pesticides is resulting in contamination/mixing of untreated wastewater with the available water resources (El-sheekh et al. 2000; Ghosh et al. 2012). The report of World Health Organization (WHO 2000) and a survey of Central Pollution Control Board, India (CPCB 2009) stated that only 31 and 35 % of the total sewage wastewater is treated up to secondary level in Asia and urban cities of India, respectively, with a capacity gap of 65–69 %. Further, the presence of excess nutrients (N and P) in untreated wastewater is resulting in



eutrophication, algal blooms, uncontrolled spread of certain aquatic macrophytes, oxygen depletion and loss of key floral and faunal species, leading to the total degradation of water bodies (Khan and Ansari 2005). Therefore, there is a need to identify cost-effective, eco-friendly technologies that require minimal infrastructure, inputs and simple know-how, which can be utilized by the common man or less literate population. These technologies should also be applicable at the small-scale level with potential of acceptance at commercial level in the future.

The methods applied in the treatment of effluents or contaminated water are broadly classified into three types—physical, chemical and biological (Fig. 1). These can be employed individually or in combination, depending upon the extent and type of pollution. In order to achieve the desired levels of contaminant removal, individual wastewater treatment procedures are grouped into a variety of systems, classified as primary, secondary and tertiary wastewater treatments. In general, both physical and chemical methods are costly. Also, most chemical methods increase the pH, conductivity and overall load of dissolved matter in the wastewater. In this respect, biological or bio-treatment of wastewater is a better option. The most common biological wastewater treatment applied in the treatment of municipal and industrial wastewaters is the use of activated sludge alone (Nyholm et al. 1996; Radjenovic et al. 2009) or in combination with algae (Gonzalez et al. 2008; Su et al. 2012a). However, problems related to dewatering and disposal of sludge have made researchers look for other alternatives.

Phytoremediation, the use of plants (including algae or lower plants) and associated microflora for the removal or biotransformation of pollutants including nutrients, heavy metals etc. from wastewater seems to be a promising option (Ali et al. 2013; Franchino et al. 2013; Richards and Mullins 2013; Sood et al. 2012). Oswald and Gotaas (1955) are the pioneers in this area, especially in terms of illustrating the potential of algae in the wastewater treatment. Oswald et al. (1957) reported designs of natural treatment systems empowered primarily by solar energy, making wastewater treatment more affordable and sustainable. Wastewater treatment with microalgae, also referred to as Phycoremediation, is a term coined recently by John (2000), as given by Souza et al. (2012). Phycoremediation is particularly attractive because it has the ability to deal with more than one problem on-site. The promising attributes of microalgae, such as (1) higher photosynthetic capabilities as compared to higher plants (Bhatnagar et al. 2011), (2) ability to convert solar energy and CO<sub>2</sub> emissions from power plants, hence, lower energy requirements (Razzak et al. 2013), (3) capacity to incorporate excess nutrients such as nitrogen and phosphorus from sewage water for their growth, making disposal easy (Bhatnagar et al. 2011; Mata et al. 2012),

(4) tolerance to extreme conditions (Makandar and Bhatnagar 2010), (5) ability to reduce greenhouse gas emissions (Bhola et al. 2014; Singh and Ahluwalia 2013), (6) wide applications of harvested biomass (Gupta et al. 2013). These useful features of microalgae have further strengthened their exploitation in wastewater treatment, as compared to the use of higher aquatic macrophytes (Table 1). Therefore, the cultivation of algae in wastewater offers the combined advantages of mitigation of greenhouse gases, treatment of the wastewaters, and simultaneously producing algal biomass. This biomass can be exploited for multiple uses—as protein supplements and food additives (animal and human feed), bioenergy resources (biogas and biofuels), bio-ore for precious heavy metals, pharmaceuticals, cosmetics and other valuable chemicals (Gupta et al. 2013; Pittman et al. 2011; Sahu et al. 2013; Singh et al. 2011; Spolaore et al. 2006).

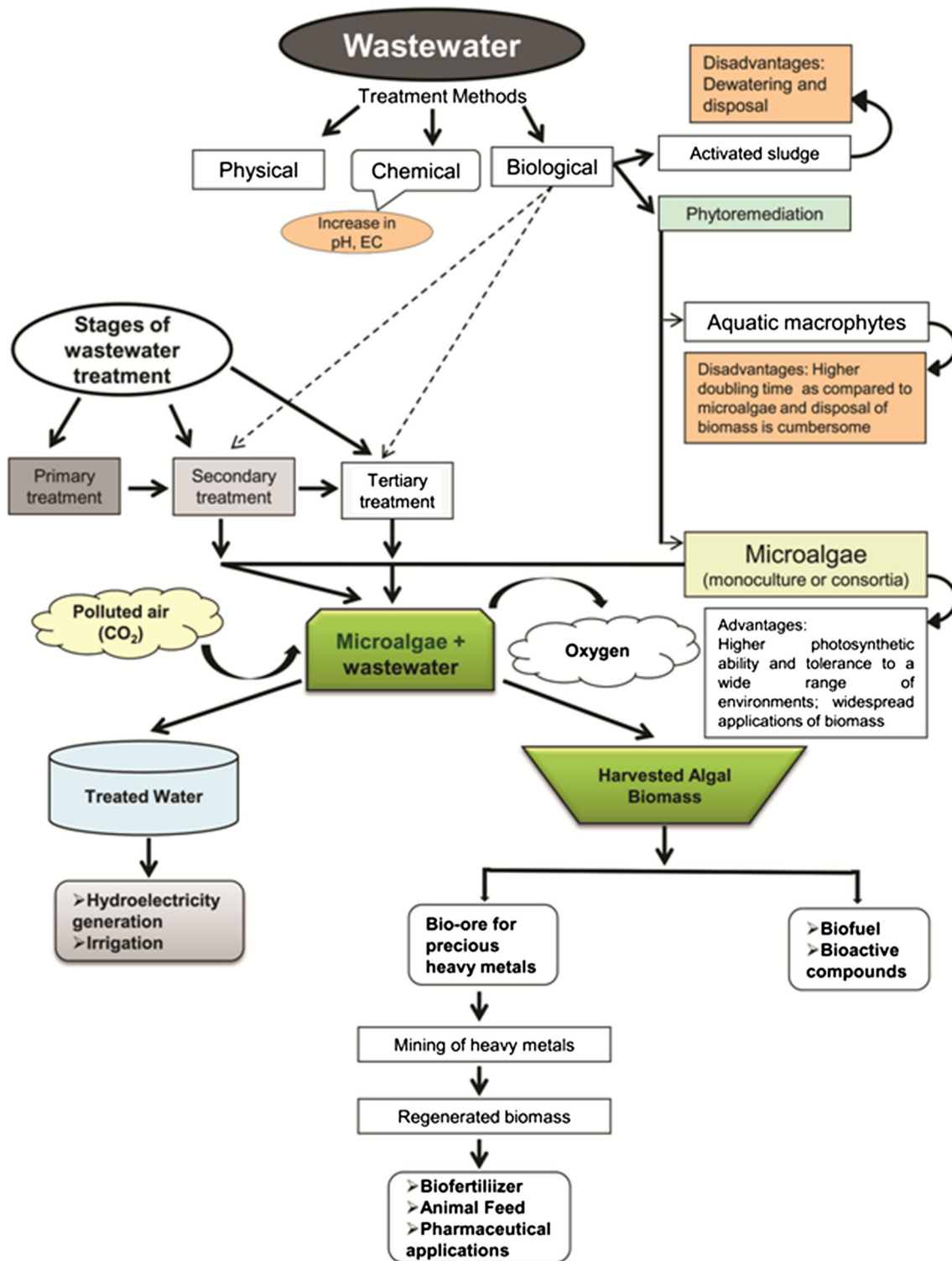
This review is, therefore, an attempt to summarize the reports available on the diversity of microalgae in various wastewaters and critically evaluate their role in wastewater treatment, besides exploring the potential of wastewaters for efficient microalgae biomass production. Since, various microalgae differ in their nutrient sequestration ability and competitive potential in different wastewaters under natural environments, the significance of consortial approach is also discussed.

#### Microalgal diversity in wastewater

The release of industrial and municipal wastewater poses serious environmental challenges to the receiving water bodies (Yang et al. 2008). Wastewater is usually rich in contaminants in the form of nutrients, heavy metals, hydrocarbons etc. The presence of nutrients especially nitrogen (N) and phosphorus (P), in the form of nitrate, nitrite, ammonia/ammonium or phosphorus in wastewater leads to eutrophication (Liu et al. 2010; Yang et al. 2008). Microalgae represent an integral part of the microbial diversity of wastewaters, which can also play a role in the self-purification of these wastewaters (Sen et al. 2013).

Microalgae constitute a broad category of organisms encompassing photoautotrophic eukaryotic microalgae and prokaryotic cyanobacteria, which are distributed both in fresh and marine environments, with a wide range of diversity in their thallus organization and habitat (Lee 2008). The biodiversity of microalgae is enormous and estimated to be about 200,000–800,000 species, out of which about 50,000 species are only described (Starckx 2012). This enormous diversity and propensity to adapt to extreme and inhospitable habitats has led the scientific community to screen, identify promising strains/species/genera and develop promising microalgae-based





**Fig. 1** Schematic representation of wastewater treatment using microalgae: overview of advantages and applications

technologies for wastewater treatment (Fouillard 2012). The available literature relevant in relation to research undertaken in terms of microalgal diversity in various types of wastewaters is summarized in Table 2.

Hussein and Gharib (2012) analyzed the phytoplankton diversity in sewage water mixed with drain water and observed a total of 152 taxa, including Bacillariophyceae (60), Chlorophyceae (20), Cyanophyceae (20), Euglenophyceae (17) and



**Table 1** Comparison of wastewater treatment potential of microalgae and higher aquatic macrophytes

Characteristics	Microalgae	Higher aquatic macrophytes
Doubling time	Microalgae double their biomass within 1–2 days	Much higher time is required by aquatic macrophytes to double their biomass
CO <sub>2</sub> sequestration potential	Much higher photosynthetic efficiency provides them with relatively higher CO <sub>2</sub> mitigation potential. Therefore, help in reduction of greenhouse effect to solve the problem of global warming	Relatively lower photosynthetic efficiency hence, lower CO <sub>2</sub> mitigation potential
Space requirement	Smaller dimensions require less space for the growth of microalgae as monoculture or consortia	Large size of macrophytes require more space for their maintenance and growth
Processing	Relatively easy to scale up of process because they can be harvested with relative ease (because of filamentous nature or flocculation ability)	Difficult to scale up process at commercial levels because of rooted nature of macrophytes
Biomass disposal and its applications	Smaller size of microalgae results in easy disposal and transport of biomass for other biotechnological applications from their site of production to their utilization sites	Lesser number of applications of biomass are explored as their huge biomass is difficult to dispose and transport

Dinophyceae (9). Bacillariophyta was the dominant group, constituting 39.4 % of overall diversity in the drain. However, in an open sewage-contaminated channel, Renuka et al. (2013a) observed the dominance (58 %) of Cyanophycean members comprising species of *Chroococcus* (Fig. 2a, b), *Lyngbya* (Fig. 2c) *Phormidium* (Fig. 2d), *Limnothrix* (Fig. 2e), *Oscillatoria* (Fig. 2f), and *Planktothrix* (Fig. 2g), followed by members of Chlorophyta (25 %) and Bacillariophyta (17 %). Bernal et al. (2008) studied the change in microalgal community in batch reactors of municipal wastewater treatment containing dairy sewage water and observed that microalgae from Cyanophyta, Chlorophyta and Euglenophyta groups were present during all the phases of the treatment process; *Arthrospira jenneri* (Cyanophyta) and *Coccomonas* sp. (Chlorophyta) were the most common members (Table 2).

In a study on the wastewater treatment plant (WWTP) at Shimoga Town, Karnataka State, India, seventy-one species belonging to Cyanophyceae, Chlorophyceae, Euglenophyceae, Bacillariophyceae and Desmidiaceae were recorded by Shanthala et al. (2009). *Chlorella* and *Scenedesmus* (Chlorophyta) were the dominant forms throughout the year, and the high pollution load was observed to have a negative impact on the total phytoplankton diversity. In another study from a wastewater stabilization pond, Furtado et al. (2009) isolated ten cyanobacterial genera as the dominant forms, including *Synechococcus*, *Merismopedia*, *Leptolyngbya*, *Limnothrix* and *Nostoc*, which represented more than 90 % of the total phytoplankton diversity of waste stabilization pond, during the periods of summer and autumn (Table 2).

Cyanobacteria also constitute an important part of the phytoplankton diversity of WWTP, due to the existence of warm, stable and nutrient-enriched water (Badr et al. 2010; Martins et al. 2010; Vasconcelos and Pereira 2001). Vasconcelos and Pereira (2001) studied the phytoplankton communities of two ponds (facultative and maturation) of the WWTP of Esmoriz (North Portugal) and reported that cyanobacteria constitute 15.2–99.8 % of the total phytoplankton diversity. Among these, *Planktothrix mougeotii*, *Microcystis aeruginosa* and *Pseudoanabaena mucicola* were the dominant species (Table 2). Similarly, in another report, Badr et al. (2010) noticed that cyanobacteria in facultative and maturation ponds of WWTP of El-Sadat city, Egypt constituted 2–97.8 % of the total phytoplankton density. Martins et al. (2010) isolated 51 strains of cyanobacteria belonging to *Phormidium autumnale*, *Planktothrix mougeotii*, *Limnothrix* sp. and *Synechocystis* sp. during a 12-month study from WWTP located in the north of Portugal. Ghosh and Love (2011) reported a high level of algal diversity comprising diatoms, green algae, cyanobacteria, Eustigmatophycean members, and unknown heterokonts using *rbcL* gene as a marker, in a wastewater treatment plant situated at Tampa, Florida and Northfield, Michigan.

Apart from wastewater treatment plants, reports are also available on the distribution and diversity of microalgae in industrial effluents (Dubey et al. 2011; Vijayakumar et al. 2007). Vijayakumar et al. (2007) observed that among the different effluents studied, cyanobacterial species comprise 93 % in sugar mill effluent, 91 % in dye effluent, and 76 and 50 % in paper mill and pharmaceutical effluents, respectively. In all these effluents, the cyanobacterial genus *Oscillatoria* was the dominant form, followed by *Phormidium*, *Lyngbya*, *Microcystis* and *Synechococcus* (Table 2). Dubey et al. (2011) recorded a total of 25 species of cyanobacteria in paper mill and pharmaceutical effluents. *Microcystis aeruginosa*, *Oscillatoria curvipes*, *O. princeps*, *Phormidium ambiguum* and

**Table 2** Microalgal diversity in selected types of wastewaters

Types of wastewater	Geographical region	Cyanobacteria	Other microalgae	References
Drain	West of Alexandria, Egypt	<i>Melosira variane</i> , <i>Cylindrotheca closterium</i> , <i>Cyclotella glomerata</i> , <i>Cyclotella kuetzingiana</i> , <i>Cyclotella meneghiniana</i> , <i>Ankistrodesmus falcatus</i> , <i>Skeletonema costatum</i> , <i>Aulacoseira distans</i> , <i>Acutodesmus acuminatus</i> , <i>Planktolingbya linnetica</i> , <i>Pseudoanabaena linnetica</i> , <i>Lyngbya contorta</i> , <i>Ankistrodesmus falcatus</i> , <i>Merismopedi punctata</i> , <i>Thalassiosira subtilis</i>	<i>Chlorella vulgaris</i> , <i>Scenedesmus bijugus</i> , <i>Euglena caudata</i> , <i>Phacus triquetra</i>	Hussein and Gharib (2012)
Municipal and dairy wastewater	Central Veracruz, Mexico	<i>Arthrospira jenneri</i> , <i>Geitlerinema</i> , <i>Synechocystis</i> , <i>Cyanobium</i> and <i>Glaucospira</i>	<i>Polytomella</i> sp., <i>Polytoma tetraolare</i> , <i>Chlamydomonas caeca</i> , <i>Carteria</i> sp., <i>Lepocynclis ovum</i> and <i>Euglena clavata</i>	Bernal et al. (2008)
Facultative waste stabilization pond	Sao Paulo State, Brazil	<i>Synechococcus</i> sp., <i>Merismopedi</i> sp., <i>Leptolyngbya</i> sp., <i>Limnothrix</i> sp., and <i>Nostoc</i> sp.	NA	Furtado et al. (2009)
Facultative and maturation ponds of wastewater treatment plant	Esmoriz, North Portugal	<i>Planktothrix mougeotii</i> , <i>Microcystis aeruginosa</i> , <i>Pseudanabaena mucicola</i> , <i>Oscillatoria</i> sp.	NA	Vasconcelos and Pereira (2001)
Wastewater treatment plant	El-Sadat city, Egypt	<i>Oscillatoria</i> spp.	NA	Badr et al. (2010)
Sugar and paper mill, dye and pharmaceutical effluent	North Portugal	<i>Phormidium autumnale</i> , <i>Planktothrix mougeotii</i> , <i>Limnothrix</i> sp. and <i>Synechocystis</i> sp.	NA	Martins et al. (2010)
Paper mill and pharmaceutical effluent	Tamil Nadu, India	<i>Oscillatoria</i> sp., <i>Lyngbya</i> sp., <i>Microcystis</i> sp., <i>Synechococcus</i> sp.	NA	Vijayakumar et al. (2007)
Pulp and paper mill effluent (secondary treatment basin)	Madhya Pradesh, India	<i>Microcystis aeruginosa</i> , <i>Oscillatoria curviceps</i> , <i>Oscillatoria princeps</i> , <i>Phormidium ambiguum</i> , <i>Phormidium corium</i>	NA	Dubey et al. (2011)
Carpet mill effluent	Brazil, Canada, New Zealand, USA	<i>Phormidium</i> , <i>Geitlerinema</i> , <i>Pseudanabaena</i> and <i>Chroococcus</i>	NA	Kirkwood et al. (2001)
	Athens, USA	<i>Anabaena</i> sp., <i>Aphanocapsa</i> spp., <i>Calothrix braunii</i> , <i>Lyngbya</i> sp., <i>Nostoc</i> sp., <i>Oscillatoria</i> sp., <i>Limnothrix</i> spp., <i>Phormidium</i> sp., <i>Chroococcus</i> sp., <i>Synechocystis</i> sp.,	<i>Chlorella vulgaris</i> , <i>Chlamydomonas</i> sp., <i>Scenedesmus</i> spp., <i>Ulothrix</i> sp., <i>Chlorococcum</i> sp., <i>Gloeocystis vesiculosa</i> , <i>Chlorococcum humicola</i> , <i>Nitzschia</i> sp., <i>Navicula</i> sp.	Chinnasamy et al. (2010a)

NA not available







**Fig. 2** Photomicrographs of dominant cyanobacterial genera commonly observed in wastewater. **a, b** *Chroococcus* sp. **c** *Lyngbya* sp. **d** *Phormidium* sp. **e** *Limnothrix* sp. **f** *Oscillatoria* sp. **g** *Planktothrix* sp. (scale bar 20  $\mu$ m)

*P. corium* were found to be common to both the effluents. They also observed *Oscillatoria* sp. as the most dominant genus. In another report, cyanobacterial communities belonging to Oscillatoriales and Chroococcales were found in the effluents of pulp and paper secondary wastewater treatment systems of Brazil, Canada, New Zealand, and USA (Kirkwood et al. 2001); *Phormidium*, *Geitlerinema*, *Pseudanabaena* and *Chroococcus* were the dominant genera. Chinnasamy et al. (2010a) assessed the microalgal diversity of treated and untreated carpet mill effluent and observed more or less equal diversity of both Cyanophyceae and Chlorophyceae members in treated wastewater during spring season, whereas Chlorophyta was the dominant group in all the seasons in untreated wastewater (Table 2).

It is now documented that algae represent a significant biotic component of such aquatic ecosystems, showing a wide range of morphological and taxonomic diversity which is significantly influenced by composition and concentration of various contaminants present. Despite the availability of a large number of reports, the range of habitats, environmental conditions and diverse types of wastewaters makes it difficult to come out with generalizations regarding diversity, especially the factors contributing to the overall qualitative and quantitative aspects of microalgal diversity. However, the following points can be concluded from Table 2; these are (1) Cyanobacteria are predominant in most types of wastewaters, followed by green algal members, and (2) the critical factors seem to be environmental conditions or different geographical locations of study areas which need in-depth and long-term investigations.

#### Use of microalgal monocultures in nutrient sequestration and biomass production

The use of microalgae for the treatment of municipal wastewater has been a subject of research for several decades (Oswald 1988). Several microalgae are efficient in the removal of nutrients (N and P) from wastewater, and many species proliferate in wastewaters due to the abundance of carbon, nitrogen and phosphorus that serve as nutrients for their growth. However, the nutrient sequestration or wastewater treatment ability of microalgae differs across genera, species and strains. Many researchers have evaluated the potential of various microalgal monocultures for nutrient removal vis-a-vis biomass production in various types of wastewaters (Tables 3, 4). Since, wastewaters differ in their physicochemical characteristics, which directly or indirectly can have an effect on algal growth; it is difficult to provide any generalization regarding the quality and quantity of generated biomass. Therefore, it is important to first check the suitability of such wastewaters for the growth of microalgal monocultures, before they can be utilized at pilot scale for biotechnological applications and other downstream processes. Such studies on screening of individual microalgae (or microalgal monocultures) also help in deciphering the potential of individual strain vis-a-vis removal of specific or several nutrients.

#### Use of municipal wastewater

Municipal/domestic wastewaters are usually rich in nutrients, mainly, nitrogen and phosphorus; enough information



**Table 3** Overview of biomass production by microalgae in selected types of wastewaters

Wastewater	Type of microalgae	Culture conditions	Mode of culture, culture volume	Biomass accumulated per day (DCW $\text{g L}^{-1} \text{ day}^{-1}$ )	References
Municipal wastewater centrate	<i>Chlorella kessleri</i> , <i>C. protothecoides</i>	Light intensity—100 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ Temperature—25 °C	Batch, 100 mL in 250-mL flask	0.402 (5), 0.262 (5)	Li et al. (2011a)
Municipal wastewater centrate	<i>Chlorella sorokiniana</i>	Light intensity—80 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ Temperature—30 °C	Batch, photobioreactor made of 1-L Duran bottle	0.055–0.083 (4)	Lizzul et al. (2014)
Municipal wastewater	<i>Chlorella</i> sp.	Light intensity—200 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ Temperature—30 °C pH—7 Photoperiod—16:8 h light dark cycle Aeration—1 % $\text{CO}_2$	NA, 300 mL in 1-L Erlenmeyer flask	0.60 (5)	Cho et al. (2013)
Municipal wastewater	<i>Chlorella vulgaris</i>	Light intensity—150 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ Temperature—20 °C Photoperiod—14:10 h light dark cycle	NA, borosilicate bioreactor	0.04–0.195 (1)	Cabanelas et al. (2013)
Secondary treated municipal wastewater	<i>Neochloris oleoabundans</i>	Light intensity—17.92 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ Aeration—5 % $\text{CO}_2$	NA, 400 mL in 500-mL cylindrical flask	0.23 (3)	Wang and Lan (2011)
Primary treated sewage and Primary treated piggery wastewater	<i>Haematococcus pluvialis</i>	Light intensity—50 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ Photoperiod—12:12 h light dark cycle Aeration—5 % $\text{CO}_2$ mixed air	NA, 130 mL in 250-mL Erlenmeyer flask	0.098–0.178 (8)	Kang et al. (2006)
Wastewater treatment plant	<i>Kirchneriella</i> sp.	Light intensity—90 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ Temperature—20 °C Photoperiod—24:4 h light dark cycle	Batch, 10 % inoculum in 5-L capacity rectangular trays	0.042 (14)	Frampton et al. (2013)
Piggery wastewater	<i>Chlorella zofingiensis</i>	Aeration—1 % $\text{CO}_2$ Light intensity—842 $\pm$ 778 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (sunlight) Temperature—29.4 °C Aeration—5–6 % $\text{CO}_2$	Semi-continuous, 720 mL in 1.37-L tubular bubble column bioreactor	1.314 (1)	Zhu et al. (2013)
Urban wastewater	<i>Desmodesmus</i> sp.	Temperature—25 °C Photoperiod—16:8 h light dark cycle Aeration—2 % $\text{CO}_2$ mixed air	Batch, 1 L Ilmabor® bottles	0.138–0.227 (1)	Samori et al. (2013)

NA not available; # values in parentheses denote number of days for cultivation



**Table 4** Nutrient removal by microalgae in different types of wastewaters

Wastewater	Type of microalgae	Culture conditions	Mode of culture, culture volume	Parameters evaluated	Initial concentration of nutrients (mg L <sup>-1</sup> )	% Removal per day	Biomass accumulated per day (DCW g L <sup>-1</sup> day <sup>-1</sup> )	References
Municipal wastewater	<i>Chlorella</i> sp.	Light intensity— $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—25 °C	NA, 100 mL in 250-mL Erlenmeyer flasks	NH <sub>4</sub> -N, total P	33.4, 5.66	9.1 (9), 10.06 (9)	NA	Wang et al. (2010a)
Municipal wastewater centrate	<i>Chlorella</i> sp.	Light intensity— $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—25 °C	Batch, 100 mL in 250-mL Erlenmeyer flasks	Total N, total P	116.1, 212	6.3 (14), 5.7 (14)	0.07 (14)	Li et al. (2011b)
Municipal wastewater centrate	<i>Chlorella</i> sp.	Light intensity— $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—26–29 °C	Batch, 300 L culture in 1,200-L centrate in pilot scale photobioreactor (10.2 cm water level)	Total N, soluble total P	290, 530	4.7 (13), 4.7 (13)	NA	Min et al. (2011)
Municipal wastewater filtered through 0.2, 1 $\mu\text{m}$ membrane and UV-B treated	<i>Chlorella</i> sp.	Light intensity— $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—25 °C Photoperiod—continuous light pH—7.2	Batch, 500 mL in 1-L Erlenmeyer flasks	Total N, total P	18.9, 1.7	8.3–10.2 (9), 9.3–9.5 (9)	0.04–0.07 (9)	Cho et al. (2011)
Domestic wastewater	<i>Chlorella vulgaris</i>	Light intensity— $56 \mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—24 °C Photoperiod—12:12 h light dark cycle	Batch, 5-L batch reactor	NO <sub>3</sub> -N, NO <sub>2</sub> -N, NH <sub>4</sub> -N, PO <sub>4</sub> -P	50.0–80.0, 18.0–25.0, 0.7–1.4, 10.0–20.0	1.52 (23), 3.26 (23), 2.17 (23), 2.6 (23)	NA	Singh and Thomas (2012)
Treated domestic wastewater	<i>Chlorella vulgaris</i> and <i>Botryococcus braunii</i>	Light intensity— $49 \mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—25 °C Photoperiod—12:12 h light dark cycle pH—7.2	NA, 1.3 L in 2-L flasks	NO <sub>3</sub> -N, PO <sub>4</sub> -P	0.20, 2.00	5.2 (14), 7.14 (14) and 5.68 (14), 7.14 (14)	0.05 (14) and 0.03 (14)	Sydney et al. (2011)
Tertiary municipal wastewater	<i>Chlorella vulgaris</i>	Light intensity— $45\text{--}50 \mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—27 °C Photoperiod—16:8 h light dark cycle	Batch, (2 % inoculum) 200 mL filter sterilized wastewater in 500-mL aluminum crimp	Total N, total P	8.7, 1.71	25 (4), 25 (4)	0.04 (5)	Ji et al. (2013)





Table 4 continued

Wastewater	Type of microalgae	Culture conditions	Mode of culture, culture volume	Parameters evaluated	Initial concentration of nutrients (mg L <sup>-1</sup> )	% Removal per day	Biomass accumulated per day (DCW g L <sup>-1</sup> day <sup>-1</sup> )	References
Wastewater treatment plant	<i>Chlorella vulgaris</i> ; <i>Scenedesmus rubescens</i> ; <i>Chlamydomonas reinhardtii</i> ; <i>Phormidium</i> sp.	Light intensity—7,000 Lux Photoperiod—12:12 h light dark cycle	Batch, 5-L glass beakers	Total N, total P	50.0, 8.8	16.5 (6), 33 (3); 16.5 (6), 24.7 (4); 24.7 (4), 49.5 (2); 14.1 (7), 24.7 (4)	NA	Su et al. (2012b)
Concentrated municipal wastewater	<i>Auxenochlorella protothecoides</i>	NA	Batch, NA	Total N, total P, total organic C	134.0, 212.0, 960.0	9.8 (6), 13.5 (6), 16 (6)	0.193 (6)	Zhou et al. (2012)
Brewery effluent	<i>Scenedesmus obliquus</i>	Light intensity— $168 \mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—30 °C Photoperiod—continuous illumination	Batch, 250 mL in Erlenmeyer flasks	Total N, total C	54.0, 2.1	1.48 (14), 4.37 (13)	0.1 (9)	Mata et al. (2012)
Piggery wastewater (filter sterilized)	<i>Chlamydomonas mexicana</i>	Light intensity— $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—27 °C	Batch, 250 mL in Erlenmeyer flasks	Total N, total P, total inorganic C	56, 13.5, 571	3.12 (20), 1.4 (20), 1.45 (20)	0.028 (20)	Abou-Shanab et al. (2013)
Industrial wastewater	<i>Chlamydomonas</i> sp.	Light intensity— $125 \mu\text{mol m}^{-2} \text{s}^{-1}$ Photoperiod—continuous illumination Aeration—5 % CO <sub>2</sub> mixed air	Batch, 5 L in 6-L glass flasks	NO <sub>3</sub> -N, NH <sub>4</sub> -N, PO <sub>4</sub> -P	16.2, 3.1, 44.7	10 (10), 10 (10), 3.3 (10)	0.134 (10)	Wu et al. (2012)
Piggery wastewater (diluted)	<i>Chlorella pyrenoidosa</i>	Light intensity— $63 \mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—25–27 °C pH—8	Batch, 500 mL in 1-L conical flask	NH <sub>4</sub> -N	34.7	9.0–9.23 (10)	0.018 (10)	Wang et al. (2012)
Digested piggery wastewater (diluted 15 times)	<i>Oedogonium</i> sp.	NA	NA	NH <sub>4</sub> -N, total P	59.4, 6.2	13.7 (7), 13.2 (7)	NA	Wang et al. (2013)
Digested dairy manure (diluted and filtered through 1.5 $\mu\text{m}$ )	<i>Chlorella</i> sp.	Light intensity— $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—25 °C Photoperiod—	10 % inoculum in 250-mL Erlenmeyer flasks	NH <sub>4</sub> -N, total N, total P	2,232.0, 3,456.0, 49.0	4.7 (21), 3.7 (21), 3.4 (21)	NA	Wang et al. (2010b)



Table 4 continued

Wastewater	Type of microalgae	Culture conditions	Mode of culture, culture volume	Parameters evaluated	Initial concentration of nutrients (mg L <sup>-1</sup> )	% Removal per day	Biomass accumulated per day (DCW g L <sup>-1</sup> day <sup>-1</sup> )	References
Soybean processing wastewater	<i>Chlorella pyrenoidosa</i>	Light intensity—40.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—27 °C Photoperiod—14:10 h light dark cycle	Fed batch culture, 500-mL conical flask	NH <sub>4</sub> -N, total N, total P	52.1, 267.1, 56.3	17.8 (5), 17.7 (5), 14.06 (5)	0.64 (1)	Hongyang et al. (2011)
Urban wastewater	<i>Chlorella vulgaris</i> ; <i>Scenedesmus obliquus</i>	Light intensity—135 $\mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—25 °C	Batch, 2.5 L in 3-L bioreactor	NH <sub>4</sub> -N	32.5	30.1 (2); 50 (2)	NA	Ruiz-Marin et al. (2010)

NA not available; # values in parentheses denote number of days for cultivation

is available on the utilization of these wastewaters as alternative growth media for microalgae (Bhatnagar et al. 2011; Frampton et al. 2013; Mutanda et al. 2011). Table 3 summarizes the reports on microalgal biomass production in different types of wastewater.

Li et al. (2011a) studied the growth potential of *Chlorella kessleri* and *C. protothecoides* in municipal wastewater centrate. The maximum biomass of 2.01 and 1.31 g L<sup>-1</sup> was obtained in 5 days from *C. kessleri* and *C. protothecoides*, respectively, and both strains also showed the ability to grow mixotrophically in centrate (Table 3). Bhatnagar et al. (2010) revealed that *C. minutissima* can grow heterotrophically in dark acidic conditions and mixotrophically over a range of organic carbon substrates in municipal wastewater. Secondary treated municipal wastewater can be used as cultivation medium for *Neochloris oleoabundans* (Wang and Lan 2011). This alga produced 2.1 and 0.68 g L<sup>-1</sup> biomass, when grown in secondary treated municipal wastewater supplemented with 70 mg N L<sup>-1</sup> and without N, respectively (Table 3). Kang et al. (2006) reported that *Haematococcus pluvialis* produced 0.78 g L<sup>-1</sup> biomass on primary treated sewage. Frampton et al. (2013) observed that *Kirchneriella* sp. was able to produce 0.60 g L<sup>-1</sup> biomass in 14 days on wastewater collected from WWTP. Interestingly, biomass production of 3.01 g L<sup>-1</sup> was obtained by growing *Chlorella* sp. in combined wastewater from digestion tank and dewatering facility (10:90 v/v, respectively) collected from WWTP at Busan, Korea, was 1.72 times higher than that with standard BG-11 medium (Cho et al. 2013). Recently, Samori et al. (2013) were able to grow *Desmodesmus* sp. in CO<sub>2</sub>-enriched urban wastewater and biomass productivity ranging from 0.138 to 0.227 g L<sup>-1</sup> day<sup>-1</sup> was recorded (Table 3). Lizzul et al. (2014) observed that *Chlorella sorokiniana* produced 0.22 g L<sup>-1</sup> of biomass in municipal wastewater centrate and supplementation of external CO<sub>2</sub> increased biomass production to 0.33 g L<sup>-1</sup>.

The reports dealing with nutrient removal potential and biomass production in wastewaters are summarized in Table 4. Wang et al. (2010a) evaluated the nutrient removal efficiency of *Chlorella* sp. in wastewater samples collected at different points of WWTP. Highest NH<sub>4</sub>-N removal of 82.4 % was observed in wastewater collected before primary settling, while highest PO<sub>4</sub>-P removal of 90.6 % was observed in wastewater after primary settling. *Chlorella* sp. was able to remove 83.2 and 85.6 % of phosphorus from the wastewater before primary settling and centrate, respectively. Min et al. (2011) also found that *Chlorella* sp. was able to remove both nitrogen (61 %) and phosphorus (61 %) from the municipal centrate (Table 4). Li et al. (2011b) recorded that *Chlorella* sp. removed 93.9, 89.1 and 80.9 % of NH<sub>4</sub>-N, total N and P, respectively, and produced 0.92 g L<sup>-1</sup> of biomass per day on raw



municipal wastewater centrate. In another report, Cabanelas et al. (2013) reported the highest productivity ( $0.195 \text{ g L}^{-1} \text{ day}^{-1}$ ) and nutrient removal (N— $9.8 \text{ mg L}^{-1} \text{ day}^{-1}$  and P— $3 \text{ mg L}^{-1} \text{ day}^{-1}$ ) rates of *Chlorella* sp. in wastewater from WWTP with N/P ratio of 2. Kim et al. (2010) observed a 50 % decrease in  $\text{NH}_4\text{-N}$  concentration by *Chlorella vulgaris* in 48 h, when grown in raw wastewater effluent. Cho et al. (2011) studied the effect of different pre-treatments—filtration ( $0.45\text{-}\mu\text{m}$ -pore-size filter) and UV treatments (over  $1,620 \text{ mJ cm}^{-2}$ ) on the nutrient removal ability of *Chlorella* sp. They reported that *Chlorella* removed 85, 92 and 74 % of total N and 84, 86 and 84 % of total P, respectively, from secondary treated municipal wastewater filtered with  $0.2\text{-}\mu\text{m}$  membrane,  $1\text{-}\mu\text{m}$  membrane or UV-B dose, respectively. The highest biomass of  $0.67 \text{ g L}^{-1}$  was produced by *Chlorella* sp. grown in filtered secondary wastewater ( $0.2 \mu\text{m}$  followed by  $1\text{-}\mu\text{m}$  membrane) and UV dose ( $0.41$  and  $0.5 \text{ g L}^{-1}$ , respectively).

Singh and Thomas (2012) compared the nutrient removal potential of different microalgae—*Chlorella* sp., *C. vulgaris*, *Scenedesmus quadricauda* and *S. dimorphus* as monocultures in permeate from aerobic membrane bioreactor fed with domestic wastewater. Among different microalgae, *C. vulgaris* was able to remove 35, 75, 50 and 60 % of  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{NH}_4\text{-N}$  and  $\text{PO}_4\text{-P}$ , respectively, after 23 days in continuous aerobic membrane bioreactor (microalgae membrane bioreactor). However, all four microalgae were able to remove 100 % of  $\text{NH}_4\text{-N}$  and 43–54, 83–95 and 70–92 % of  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$  and  $\text{PO}_4\text{-P}$ , respectively, after 3 days in the batch reactor (Table 4). Ji et al. (2013) studied the biomass production and nutrient removal potential of *Chlorella vulgaris*, *Scenedesmus obliquus* and *Ourococcus multisporus* in tertiary treated municipal wastewater. All the studied microalgae removed more than 99 % of the nitrogen and phosphorus in 4 days. Biomass production of  $0.197 \text{ g L}^{-1}$  was obtained by *Chlorella vulgaris*, *Scenedesmus obliquus*, while *Ourococcus multisporus* accounted for  $0.203 \text{ g L}^{-1}$  of biomass in tertiary wastewater effluent (Ji et al. 2013).

Nutrient removal potential of 13 microalgal strains in treated domestic wastewater was determined by Sydney et al. (2011). They reported that *Botryococcus braunii* removed 79.63 % of the nitrogen and 100 % phosphorus from treated domestic wastewater in 14 days. Su et al. (2012b) observed that *Phormidium* sp., *Chlamydomonas reinhardtii*, *Chlorella vulgaris* and *Scenedesmus rubescens* removed 99 % of total N within 7, 4, 6 and 6 days, and P in 4, 2, 3 and 4 days, respectively, from the effluent collected from wastewater treatment plant of Holthusen, Germany. Zhou et al. (2012) reported that *Auxenochlorella protothecoides* was able to remove 59, 81 and 96 % of total N, P and C, respectively, when grown in concentrated municipal

wastewater for 6 days. This microalga produced  $1.16 \text{ g L}^{-1}$  of biomass in 6 days in the same medium (Table 4).

#### Other wastewaters

Apart from municipal wastewater, Kang et al. (2006) reported that *Haematococcus pluvialis* can also produce  $1.43 \text{ g L}^{-1}$  biomass, when grown in diluted (fourfold) primary piggery wastewater (Table 3). Zhu et al. (2013) recorded biomass productivity of  $1.314 \text{ g L}^{-1} \text{ day}^{-1}$  by cultivating *Chlorella zofingiensis* in piggery wastewater pre-treated with sodium hypochlorite ( $\text{NaClO}$ ). Ryu et al. (2013) found a biomass productivity of  $6.69 \text{ g L}^{-1} \text{ day}^{-1}$  for *Aurantiochytrium* sp., using spent yeast from brewery industry as the growth substrate, with simple stirring as pre-treatment.

Mata et al. (2012) studied the growth and wastewater treatment potential of *Scenedesmus obliquus* in brewery effluent. The alga was able to remove 20.8 % of total N and 56.9 % of total carbon in 9 and 13 days, respectively. This microalga produced  $0.9 \text{ g L}^{-1}$  of biomass in 9 days (Table 4). Abou-Shanab et al. (2013) screened different microalgal strains viz. *Ourococcus multisporus*, *Nitzschia cf. pusilla*, *Chlamydomonas mexicana*, *Scenedesmus obliquus*, *Chlorella vulgaris*, and *Micractinium reisseri* for nutrient removal and biomass production in piggery wastewater. Highest removal of total N, P and inorganic C of 62, 28 and 29 % was obtained by *C. mexicana*. This alga also produced highest dry biomass of  $0.56 \text{ g L}^{-1}$  compared to other microalgae grown in piggery wastewater for 20 days. *Chlamydomonas* sp. removed 100 % of  $\text{NO}_3\text{-N}$ ,  $\text{NH}_3\text{-N}$  and 33 %  $\text{PO}_4\text{-P}$  and produced  $1.34 \text{ g L}^{-1}$  biomass from the industrial effluent in 10 days (Wu et al. 2012). In another report by Lim et al. (2010), *Chlorella vulgaris* removed 45.1 % of  $\text{NH}_4\text{-N}$ , 33.3 %  $\text{PO}_4\text{-P}$  and produced  $0.107 \text{ g L}^{-1}$  biomass in textile wastewater. They observed that the addition of nutrients of Bold's Basal medium to textile wastewater increased the biomass production to  $0.613 \text{ g L}^{-1}$ , however, did not improve the removal of nutrients. Ruiz-Marin et al. (2010) compared the N and P removal ability of immobilized and free cells of *Chlorella vulgaris* and *Scenedesmus obliquus*, and found that *S. obliquus* had a higher N and P uptake in urban wastewater than *Chlorella vulgaris*. However, *S. obliquus* can be more effective in nutrient removal when immobilized with the recalcification of beads than free cell cultures (Table 4). Free cells of *Chlorella vulgaris* can remove 60.1 % of ammonia in 48 h in urban wastewater, while 100 % of ammonia removal was observed with *Scenedesmus obliquus* in the same time duration. Gonzalez et al. (2008) reported that *Chlorella sorokiniana* culture mixed with activated sludge removed 86 and 87 % of total



organic carbon and  $\text{NH}_4\text{-N}$  from four times diluted pre-treated piggery wastewater.

From these studies (Tables 3, 4), it can be summarized that *Chlorella* is the most explored microalga for the removal of nutrients (N and P) from different types of wastewaters and biomass production. A huge variation in the percent removal of nutrients with *Chlorella* spp. has been observed in the published literature (Tables 3, 4). Biomass productivity of  $0.03\text{--}1.314\text{ g L}^{-1}\text{ day}^{-1}$  was recorded using various *Chlorella* spp. Highest biomass productivity of  $1.314\text{ g L}^{-1}\text{ day}^{-1}$  was obtained from *Chlorella zofingiensis* grown in sunlight, in a bioreactor running in semi-continuous mode supplied with 5–6 %  $\text{CO}_2$  in piggery wastewater (Table 3). The observed variation in the nutrient removal potential and biomass productivity of various microalgal species can be attributed to not only the differences in the culture conditions (viz. light intensity, temperature, photoperiod, mode of culture) but also the type of wastewater used. The composition and concentration of nutrients and other harmful contaminants present in the wastewaters, the habitat of the strain, nature (biochemical attributes or genetic potential) of strain/species used are other important factors.

Despite many recent reports on utilization of microalgae in wastewater treatment and biomass generation, the problems related to harvesting of unicellular, monocultures of microalgae due to their microscopic dimensions have restricted its commercialization. Therefore, filamentous forms (with comparatively large dimensions) or consortial approach are being explored as more viable options to reduce the overall cost of the process and obtain better harvesting rates.

#### Significance and promise of consortial approach

No organism can sustain in totality in any ecosystem, as is illustrated by the above studies in which the monoculture of different microalgae need to compete with the indigenous microflora present in different wastewaters, which in turn, play a critical role in the overall biomass production and wastewater treatment potential (Bernal et al. 2008; Renuka et al. 2013a). Therefore, it can be envisaged that the use of consortia can be a promising alternative to increase the efficiency of process either for biomass production or wastewater treatment (Chinnasamy et al. 2010a; Mustafa et al. 2012). However, interactions among partners and sustainability of non-native microalgae in consortial approach are the major points of concern. In consortia, one strain of microalga may be efficient in removing one type of contaminant, and the other strain can be more effective in the uptake of another (Chinnasamy et al. 2010a). Hence, the development of consortia using promising strains can help to resolve not only the problem of their harmonious

growth under unfavorable environments, but also prove synergistic and more effective in treating the wastewater in totality. Therefore, evaluation of the efficiency of native/non-native consortia either for biomass production or wastewater treatment can be promising aspects for further research in this area.

#### Wastewater treatment using microalgal consortia

The reports on wastewater treatment and biomass production by microalgal consortia are summarized in Table 5.

#### Municipal wastewater

Ruiz-Martinez et al. (2012) observed that the mixed microalgae (Chlorococcales and Cyanobacteria) isolated from the walls of the secondary clarifier in Carraixet WWTP grown in effluent of a submerged anaerobic bioreactor removed 67.2 %  $\text{NH}_4\text{-N}$  and 97.8 % of  $\text{PO}_4\text{-P}$  in 42-day cultivation period and produced  $0.234\text{ g L}^{-1}\text{ day}^{-1}$  biomass. Silva-Benavides and Torzillo (2011) compared the nutrient removal by *Chlorella* and *Chlorella-Planktothrix* co-cultures grown in municipal wastewater. *Chlorella* sp. was able to remove 100 % of  $\text{PO}_4\text{-P}$  in 2 days when grown as monoculture or co-culture with *Planktothrix*. However, about 80 % (highest) removal of nitrogen can be obtained with unshaken co-culture of *Chlorella* and *Planktothrix* from municipal wastewater (Table 5). Renuka et al. (2013b) compared the potential of consortia of native filamentous strains, native unicellular strains and selected non-native microalgae for nutrient removal potential, water quality improvement and biomass production using primary treated sewage water. This study revealed that the consortium of filamentous isolates from primary treated sewage water proved most promising in nutrient removal efficiency, and also led to higher biomass production, as compared to the other consortia employed. The consortium of filamentous strains removed 90, 100 and 97 % of  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$  and  $\text{PO}_4\text{-P}$ , and produced  $1.07\text{ g L}^{-1}$  biomass on 10th and 6th day, respectively, in the sewage wastewater.

#### Other wastewaters

Singh et al. (2011) found that the consortium of *Chlorella minutissima*, *C. sorokiniana* and *Scenedesmus bijuga* produced  $0.371\text{ g L}^{-1}$  biomass in 6 % poultry litter effluent. They suggested that mixotrophic algae can prove to be suitable candidates for large-scale wastewater treatment, with concomitant production of renewable feedstock for animal feed and bioenergy applications. In another report, Bhatnagar et al. (2011) studied the growth potential of combinations of different native strains of green



**Table 5** Biomass production and remediation potential of different microalgal consortia in various types of wastewater

Wastewater	Type of microalgal consortia	Culture conditions	Mode of culture, culture volume	Parameter undertaken	Initial nutrient concentration (mg L <sup>-1</sup> )	% Removal per day	Biomass accumulated per day (DCW g L <sup>-1</sup> day <sup>-1</sup> )	References
Municipal wastewater	Co-culture of <i>Chlorella</i> sp. and <i>Planktothrix</i> sp.	Light intensity—20–60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ pH—7 Aeration—3 % CO <sub>2</sub> mixed air	Batch, 250 mL in 500-mL flask	PO <sub>4</sub> -P, total N	7.41, 79.3	25 (4), 20 (4)	0.066 (1)	Silva-Benavides and Torzillo (2011)
Sewage wastewater	Consortium of native filamentous strains	Outdoor conditions Light intensity—420–1,760 $\mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—17–36 °C	Batch, 800 mL in 1,000-mL beaker	NO <sub>3</sub> -N, NH <sub>4</sub> -N, PO <sub>4</sub> -P	83.71, 21.06, 3.14	9.0 (10), 10.0 (10), 9.7 (10)	0.178 (6)	Renuka et al. (2013b)
Poultry effluent digester effluent (6 %)	Consortium of <i>Chlorella minutissima</i> , <i>C. sorokiniana</i> and <i>Scenedesmus bijuga</i>	Light intensity—75–80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ Temperature—25 °C Photoperiod—12:12 h light dark cycle	Batch, 60 mL in 250-mL Erlenmeyer flask	Total N, total P	114.0, 9.0	NA	0.031 (12)	Singh et al. (2011)
Treated carpet mill effluent	Consortium of native microalgae from wastewater	Light intensity—75–80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ Aeration—6 % CO <sub>2</sub> levels	Batch, 500 mL in 1-L Erlenmeyer flask	NO <sub>3</sub> -N, NH <sub>4</sub> -N, PO <sub>4</sub> -P	1.39–3.91, 0.57–3.61, 17.59–21.95	99.7 (1), 100 (1), 75 (1)	0.163 (9)	Chinnasamy et al. (2010a)
Carpet industry effluent	Consortium of <i>Chlamydomonas globosa</i> , <i>Chlorella minutissima</i> and <i>Scenedesmus bijuga</i>	Outdoor conditions Aeration—5–6 % CO <sub>2</sub> and air mixture	Batch, 20 L in polybag (95 cm deep, 15 cm diameter)	NA	NA	NA	0.07 (1)	Chinnasamy et al. (2010b)
Fish processing wastewater	Consortium of native microalgae	Light intensity—12,000 Lux Temperature—31 °C	Continuous, 3 L in photobioreactor (17 cm wide, 27 cm long, 11 cm high)	Soluble P	10.7	14.4–14.8 (5)	0.111 (1)	Riano et al. (2011)
Landfill leachate	Consortium of <i>Chlorella vulgaris</i> , <i>Scenedesmus quadricauda</i> , <i>Euglena gracilis</i> , <i>Ankistrodesmus ovalis</i> and <i>Chlorococcum oviforme</i>	Outdoor conditions	Continuous, 40 L with 0.27 m <sup>2</sup> surface area in a high rate algal pond	NH <sub>4</sub> -N, PO <sub>4</sub> -P	151.66, 8.18	NA	0.016 (351)	Mustafa et al. (2012)

NA not available; # values in parentheses denote number of days for cultivation





microalgae *Chlamydomonas globosa*, *Chlorella minutissima* and *Scenedesmus bijuga* in various types of industrial wastewaters such as—poultry litter extract, treated and untreated carpet mill effluent. They concluded that microalgae grown in poultry litter extract produced higher biomass compared with the standard growth medium BG 11. They recorded highest biomass production of  $0.349 \text{ g L}^{-1}$  in poultry litter extract and  $0.366 \text{ g L}^{-1}$  in untreated carpet mill wastewater with mixture of *Scenedesmus bijuga*–*Chlorella minutissima* culture. However, under mixotrophic conditions, the algal consortium of *Chlorella globosa*–*Chlorella minutissima* and *Scenedesmus bijuga*–*Chlorella minutissima* generated higher biomass ( $3.017$  and  $1.655 \text{ g L}^{-1}$ , respectively) in poultry litter extract and carpet wastewater, respectively. Chinnasamy et al. (2010a) observed higher growth rate and nutrient removal by the consortium of 15 native algal strains isolated from carpet mill effluent, and reported better performance of consortium in the treated wastewater, despite the low concentration of nitrogen and phosphorus. They reported removal to the tune of 99, 100 and 75 % of  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$  and  $\text{PO}_4\text{-P}$  in the first 24 h, respectively, when supplied with 6 %  $\text{CO}_2$ -enriched air at  $25^\circ\text{C}$ . The consortium was able to produce  $1.47 \text{ g L}^{-1}$  biomass in 9 days. Biomass productivity of  $0.07 \text{ g L}^{-1} \text{ day}^{-1}$  was obtained by cultivating the consortium of *Chlamydomonas globosa*, *Chlorella minutissima* and *Scenedesmus bijuga* in polybags with untreated carpet industry effluent (Chinnasamy et al. 2010b). In another study, a consortium of microalgae isolated from a lagoon containing aerobically treated swine slurry was helpful in the efficient removal of nutrient from fish processing wastewater, recording 70 % removal of phosphate from the wastewater and biomass productivity of  $0.111 \text{ g L}^{-1} \text{ day}^{-1}$  was achieved (Riano et al. 2011). Microalgal consortium of five microalgae viz. *Chlorella vulgaris*, *Scenedesmus quadricauda*, *Euglena gracilis*, *Ankistrodesmus convolutus* and *Chlorococcum oviforme* was able to remove 99.9 and 86 % of  $\text{NH}_4\text{-N}$  and  $\text{PO}_4\text{-P}$  from landfill leachate, when grown in high rate algal pond, producing biomass of  $5.54 \text{ g L}^{-1}$  (Mustafa et al. 2012).

The comparison between the nutrient removal potential and biomass productivity of different monocultures and microalgal consortia used in various studies revealed a higher nutrient removal rate in microalgal consortia inoculated wastewater as compared to monocultures (Table 3, 4, 5). Highest  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$  and  $\text{PO}_4\text{-P}$  removal of 99.7, 100 and 75 %, respectively, within 24 h was recorded from a consortium of native strains grown under batch mode with 6 %  $\text{CO}_2$  supply in treated carpet mill effluent (Table 5). Higher nutrient removal rate in microalgal consortia inoculated wastewaters could be due to the fact that the one microalgal strain can have high potential for removing one type of nutrient efficiently, while the other

strain can be more effective in the uptake of another. This reveals a synergistic effect in the uptake of nutrients in consortial approach. However, the biomass productivities in batch cultures were comparable in monocultures and microalgal consortia grown under different and/or close culture conditions (Tables 3, 4, 5), and sometimes lower in consortial approach. The biomass productivities of  $0.03\text{--}0.60$  and  $0.03\text{--}0.178 \text{ g L}^{-1} \text{ day}^{-1}$  were obtained in monocultures and microalgal consortia, respectively, grown in wastewater under batch mode in different investigations (Tables 3, 4, 5). Higher biomass productivity was observed in continuous and/or semi-continuous mode, as compared to batch mode of culture using monocultures (Tables 3, 4). In consortial approach, however, the biomass productivities did not differ significantly (Tables 3, 4, 5). Therefore, this area needs in-depth research regarding the interactions among the consortial members in order to enhance biomass productivity and nutrient removal.

#### Heavy metal removal by microalgae in wastewater

The presence of heavy metals and other compounds viz. phenols and detergents (Aonghusa and Gray 2002; Petrovic et al. 2003; Wang et al. 2010a) in municipal wastewater along with the nutrients is a matter of concern, because microalgae can sequester these compounds (Mani and Kumar 2014; Nacorda et al. 2007; Richards and Mullins 2013; Wang et al. 2010a). These heavy metals interfere with the uptake of macronutrients, as a result of common transporters (Levy et al. 2005; Thiel 1988). However, if present in trace amounts, some of these metals (Cu, Cd, Pb etc.) may act as micronutrients for the growing microalgae. Therefore, complete knowledge of wastewater characteristics is essential before using them as cultivation media for microalgae (Renuka et al. 2014). Apart from this, the cultivation of microalgae in heavy metals contaminated wastewaters proves problematic for the applications of biomass for food grade purposes e.g., feed, pharmaceuticals; however, the generated biomass can be used for such applications after the retrieval of heavy metals from biomass.

Ajayan et al. (2011) observed the highest Cu, Zn and Co removal of 60, 42.9 and 29.6 %, respectively, with *Oscillatoria quadripunctutata*, while highest Pb removal of 34.6 % was observed with *Scenedesmus bijuga* in sewage wastewater. In another report, *Chlorella* sp. removed 65.4, 95.4, 98.3, 80, 98.2 and 56.5 % of Al, Ca, Fe, Mg, Mn and Zn, respectively, from municipal wastewater (Wang et al. 2010a). El-Sheekh et al. (2005) also studied the removal of heavy metals from Verta Company, involved in paper production, sewage wastewater and salt and soda company wastewater by *Nostoc muscorum*, *Anabaena subcylindrica*



and mixed culture of *Nostoc muscorum* and *Anabaena subcylindrica* and found significant removal of heavy metals from all types of wastewaters using microalgae. Monocultures of *Nostoc muscorum* and *Anabaena subcylindrica* were able to remove 64.4, 22.20, 84.6 and 64.10 % and 33.3, 33.3, 86.2 and 40 % of Cu, Co, Pb and Mn, respectively, from sterilized sewage wastewater, while 75, 11.8, 100 and 61.5 % removal of Cu, Co, Pb and Mn was observed with mixed culture of *Nostoc muscorum* and *Anabaena subcylindrica* from sewage wastewater (El-Sheekh et al. 2005). This report revealed the superiority of microalgal consortium/mixed culture in the removal of Cu and Pb from wastewater, over the use of microalgal monocultures.

#### Water quality improvement by microalgae in wastewater

Microalgae are presumed to have role in self-purification of natural water bodies (Sen et al. 2013). The efficiency of microalgae in water quality improvement in wastewater has been documented in various studies (Bernal et al. 2008; Riano et al. 2011). Bernal et al. (2008) demonstrated that native microalgae growing in wastewater treatment plant removed 88, 97.3, 88.6, 91.4 and 99.9 % of chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solid (TSS), turbidity, fecal coliforms, respectively, from dairy sewage water in 25 days. Mata et al. (2012) studied the efficiency of *Scenedesmus obliquus* for the treatment of brewery effluent and revealed 20.8 % removal of COD in 14 days. *Chlorella* sp. removed 24.8 % of COD in 9 days from secondary treated municipal wastewater filtered through 0.2- $\mu\text{m}$  membrane (Cho et al. 2011). Wang et al. (2010b) revealed that *Chlorella* sp. removed 34.3 % of COD in 21 days from dairy manure effluent diluted and filtered (1.5  $\mu\text{m}$ ). Various studies illustrated 70–90 % removal of COD from municipal wastewater centrate in 9–14 days by using *Chlorella* sp. (Li et al. 2011b; Min et al. 2011; Wang et al. 2010a). *Anabaena variabilis* and *A. oryzae* removed 89.2 and 73.7 % of BOD and COD, respectively, in 7 days from domestic to industrial wastewater. However, Qun et al. (2008) revealed that an algal biofilm removed 97.1 % of COD in 6 days from simulated wastewater; while, a consortium of native microalgal strains removed 70 % of the COD in 5 days from fish processing wastewater (Riano et al. 2011). These studies indicated that various microalgae are efficient in water quality improvement in wastewater and the use of native strains and/or consortial approach may be useful for the improving the treatment process; however, the process also depends upon the culture conditions provided and the extent of contaminants.

#### Future outlook

Although, the idea of using microorganisms in bioremediation dates back to 1980, the refinement of tools and development of methods and their applications are more recent. However, several gaps still remain, which need in-depth research; among which some important areas include:

- Studies on the indigenous diversity of microalgae inhabiting different wastewaters need to be complemented using molecular tools to understand the qualitative and quantitative changes in diversity with time and treatment.
- Selection and development of different types of consortia, which can acclimatize and treat wastewater generated from diverse sources. In this respect, consortia generated with native microalgae will be more beneficial, as shown in available reports.
- Consortial approach with wider biological spectrum i.e., consortia of microalgae with bacteria or fungi may further strengthen the sustenance or competitive ability of consortia, especially using native microorganisms. This can also reduce the cost of process as they can induce self-flocculation, aiding in harvesting of these consortia. However, the presence of associated pathogenic or toxic microbes should be looked into, before evaluating their ultimate use as animal feed.
- Research in the area of metagenomics is required with an emphasis to develop and evaluate the techniques/protocols for assessment of establishment and effectiveness of inoculated versus native microalgae at the contaminated sites.
- Understanding the mechanisms of nutrient uptake and their interaction/interference by other contaminants such as heavy metals is also an important area, which is required for the success of consortial approach at commercial level.

#### Conclusion

The available literature in reports have clearly emphasized and proved beyond doubt that microalgae are efficient in nutrient removal from different types of wastewaters and can be explored for the remediation of these contaminated sites. The response and growth of different types of microalgae in wastewater also vary, because of differences in their inherent ability, especially nutrient uptake; tolerance to harsh/extreme environmental conditions and competitive potential, *vis-a-vis* indigenous organisms. Further, the difficulties encountered with the use of monocultures of microalgae, such as growth in diverse environments and



harvesting problems, highlight that the consortial approach may be a more effective alternative for wastewater treatment. Such consortia, especially showing synergistic interactions would have wider potential in treating different types of wastewater, than microalgal monocultures. The formulation of consortia of native strains can strengthen the acceptability and wider use of phycoremediation at the industrial/commercial scale. As algae represent the base of food pyramids and primary consumer in food chains, their deployment needs to be an integral part of wastewater remediation in the global scenario, as an environment-friendly strategy.

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## Abbreviations

WHO	World Health Organization
CPCB	Central Pollution Control Board
WWTP	Wastewater Treatment plant

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