Feasibility of Microalgal Technologies in Pathogen Removal from Wastewater

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1 Background

Wastewater can be defined as raw, untreated, spent water which can potentially pollute the environment. Wastewater contains impurities that were present either originally or are added by anthropogenic activities. Wastewater cannot be discharged to the receiving water body, which may be a river, lake, or sea, unless they have been treated to reduce the concentration of polluting substances to safe levels. Wastewater can originate from many sources such as homes, businesses, and industries. The source of wastewater determines its characteristics and the treatment process that wastewater should undergo. The entire wastewater treatment process involves primary, secondary, and tertiary stages which constitute physical, chemical, and biological processes. Due to the insufficiency of these processes to remove pathogens from wastewater, microalgae-mediated wastewater treatment, phycoremediation, is another paradigm for wastewater treatment. Phycoremediation involves the utilization of algae for the removal of contaminants from wastewater. Coliforms, heavy metals, and xenobiotics are effectively removed by phycoremediation, and this reduces the chemical and biological oxygen demand of wastewater (Olguín et al. 2003; Rawat et al. 2011; Abdel-Raouf et al. 2012; Cai et al. 2013). Microalgae-mediated wastewater treatment is advantageous over conventional techniques in terms of better pathogen removal, decreased sludge formation, reduced greenhouse

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gas emission, and parallel generation of energy-rich algal biomass (Cai et al. 2013; Batista et al. 2015). This chapter furnishes an overview of conventional processes and the applicability of microalgae-mediated pathogen removal from wastewater.

2 Wastewater

An insight into the characteristics of wastewater is crucial for determining the type of treatment it requires. Industries (industrial wastewater) and household activities (domestic wastewater) are majorly responsible for wastewater generation. Centralized sewage treatment plants (STPs) collect wastewater through sewage systems (underground sewage pipes), and STPs are the sites where sewage water is treated.

2.1 Wastewater Types: The two common types of wastewaters are briefed below.

Industrial Wastewater

It can be segregated into two classes as follows:

Inorganic Industrial Wastewater: It is generally produced by coal and steel industry and comprises huge amount of suspended matter. It also consists of harmful solutes like cyanides. Due to the extremely harmful nature of the effluent, these industries are so situated that they discharge their wastewater directly into municipal wastewater system after treating the effluent, in compliance with local regulations (Shi 2009).

Organic Industrial Wastewater: It contains organic waste flow from chemical industries using organic substances. This sort of wastewater is majorly produced by tanneries, leather factories, textile industries, paper manufacturing factories, oil refineries, breweries and industries manufacturing pharmaceuticals, cosmetics, organic dyes, soaps, detergents, pesticides, and herbicides. Due to the myriad of manufacturing processes, the type of effluent varies widely.

Domestic/Residential Wastewater

Domestic wastewater is generated in the residencies like houses, hotels, restaurants, offices, schools, theaters, shopping centers, commercial laundries, etc. This kind of wastewater is less toxic than industrial wastewater, and the effluent generated is also less varied as compared to industrial wastewater.

2.2 Wastewater Characterization

Physical Characteristics

Color: Fresh wastewater is usually slight gray, while septic sewage is dark gray or black. Industrial wastes containing coloring substances may affect the color of the wastewater.

Odor: Fresh wastewater has a distinctive disagreeable odor. Industrial wastewater may also add up to the odor of the wastewater by the dissemination of odorous compounds or compounds that produce odors during the process of wastewater treatment. Hydrogen sulfide is commonly responsible for the wastewater odor. The fear of generation of potential odors during treatment is so intense that implementation of wastewater treatment can be stalled.

Solids: Total solids are the total residues left after evaporation at 105 °C. Suspended solids constitute a major part of total solids and are removed from by membrane filtration. Suspended solids increase turbidity and silt load in the receiving water (Muttamara 1996).

Temperature: Geographic location governs the average temperature of wastewater. The temperature of wastewater affects chemical and biological reaction rates. Undesirable planktonic species and fungi grow fast at higher temperatures. At the same time, the effectiveness of treatment decreases at low temperatures (Muttamara 1996).

Chemical Characteristics

Organic materials: The main organic constituents in wastewater are proteins (40–60%), carbohydrates (25–50%), and fats and oils (10%) (Muttamara 1996). Urea is another key organic compound present in wastewater. The presence of easily biodegradable organic materials reduces the oxygen demand, and the presence of non-biodegradable organic material impedes the wastewater treatment processes.

Inorganic materials: Chloride, nitrogen, phosphorus, sulfur, and heavy metals are the regular inorganic constituents present in wastewater. Phosphorus is present in appreciably lower concentrations than nitrogen or carbon in natural waters. Wastewater organisms are adversely affected by the trace concentrations of inorganic materials, as these substances limit the growth of organisms present in water. The inorganics can be efficiently utilized by algae, and macroscopic plant forms their metabolism.

Gases: Nitrogen, oxygen, carbon dioxide, hydrogen sulfide, ammonia, and methane are the major gases which constitute wastewater. The maintenance of aerobic state is essential in order to annihilate problematic conditions in the wastewater treatment technology and in the natural waters receiving the effluent (Muttamara 1996). However, in anaerobic system, oxidation is carried out by the reduction of inorganic salts like sulfates or through the action of methane-forming bacteria.

Biological Characteristics

Bacteria: Wastewater makes an ideal medium for growth of both aerobic and anaerobic microbes. Among the numerous types of bacteria in wastewater, the most common types are fecal coliforms, which originate in human intestines and travel via human discharges. *Acinetobacter*, *Clostridium*, *Aeromonas*, *Enterococcus*, *Campylobacter*, *Enterobacter*, *Klebsiella*, *Escherichia*, *Mycobacterium*, *Shigella, Pantoea*, *Serratia*, *Staphylococcus*, *Salmonella*, *Pseudomonas*, and *Vibrio* are the most prevalent bacterial species in wastewater (Korzeniewska 2011). The bacteria are the key to the biological unit processes. In the presence of adequate dissolved oxygen, the soluble organic matter is converted to new cells and inorganic elements which act as substrates for higher orders of living beings, thus causing a decline in the organic loading.

Viruses: Viruses found in human excreta are a major public health hazard and enter the water stream via fecal contamination. Pathogenic viruses that majorly exist in wastewater are polio and hepatitis. Huge amount (10,000–100,000) of infectious particles of viruses are discharged per gram of feces from hepatitis-positive patients. The titer of plant and animal viruses in wastewater is comparatively small though bacterial viruses may be present (Akpor et al. 2014; Okoh et al. 2007; Gomez et al. 2000; Toze 1997). Most of the viruses are persisters and are resistant to treatment processes.

Fungi: A number of filamentous fungi are found naturally in wastewater as spores or vegetative cells. Various fungi are reported to have the ability to break down organic matter and adsorb the suspended solids in wastewater through their hyphae (Molla et al. 2004; Akpor et al. 2014). *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, and *Trichoderma* are some fungi commonly found in wastewater (Eva 2011).

Protozoa: The presence of pathogenic protozoa in wastewater is comparatively higher than other environmental sources. *Giardia intestinalis*, *Entamoeba histolytica*, and *Cryptosporidium parvum* are the prevalent protozoans, frequently detected in wastewater due to fecal contamination. Some protozoa, which are obligate aerobes, are able to survive up to 12 h in anoxic conditions and are thus excellent indicators of an aerobic environment.

Helminths: Helminths are usual intestinal parasites which, like protozoans, are spread by fecal-oral route. Wastewater is highly contaminated with these nematodes and tapeworms. Intestinal nematodes have been reported by the World Health Organization (WHO) as the most health risk comprising aquacultural/agricultural utilization of wastewater and untreated excreta (WHO 1989).

3 Conventional Technologies for Wastewater Treatment

For reuse of wastewater, nutrient conservation and pathogen removal are essential steps. The pathogen profile of wastewater varies widely with the type of wastewater (Jiménez 2003). Therefore, choice of treatment process is critically dependent on the type of wastewater (Mohiyaden et al. 2016). Various wastewater treatment stages include preliminary, primary, secondary, and tertiary treatment (Shrestha 2013; Topare et al. 2011) (Fig. 1), and every stage comprises of physical, chemical, and biological treatment processes separately or in association. A brief discussion of each of these treatment stages is given below:

Preliminary Treatment

This step removes large solids, abrasive grit, rags, and high levels of organic content (Mohiyaden et al. 2016). In preliminary treatment, bars placed at 20–60 mm are used for removing large floating objects, and retained substances are raked from the bars periodically (Tebbutt 1983). Abrasive grit material is removed by reduction in the flow speed to the level of 0.2–0.4 m/s at which sediment will settle but organic material remain suspended (Gray 1989). However, this step does not affect pathogen and nutrient concentration (Jiménez et al. 2010).

Primary Treatment

After the preliminary treatment, wastewater is treated in primary settling tanks where BOD is decreased by 40% in the form of settable solids (Horan 1990). For the partial reduction of suspended solids and organic matter, physical unit

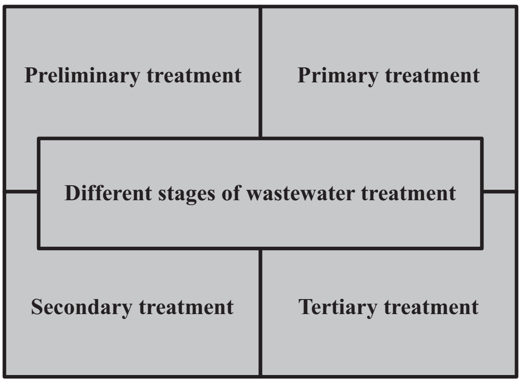

Fig. 1 Stages of wastewater treatment. (Source: Shrestha 2013; Topare et al. 2011)

operations such as sedimentation and screening or some chemicals are primarily used in primary treatment (Mohiyaden et al. 2016). In this step suspended solids (70%), BOD_5 (50%), grease and oil (65%), heavy metals, some organic nitrogen, and phosphorus are removed. The effluent leaving the primary sedimentation unit is called primary effluent (FAO 2006).

Secondary Treatment

After this, wastewater is subjected to secondary treatment for the elimination of solubilized, suspended, and colloidal matter through various biological approaches such as lagoon system, fixed-film reactors, activated sludge, etc. In this step, wastewater is treated in reactor succeeded by treatment in a secondary sedimentation tank where separation of biomass produced by the oxidation of organic matter occurs (Jiménez et al. 2010). A significant decline in BOD takes place by reduction of organic matter mediated by consortium of heterotrophic bacteria (Abdel-Raouf et al. 2012). Many workers have found that about 90% of pathogenic bacteria can be eliminated by this treatment and viruses are removed by adsorption, but rate of removal varies with the type of the reactor (Gray 1989; Kott et al. 1974; Lloyd and Morris 1983).

Tertiary/Advanced Wastewater Treatment

In this advance stage of wastewater treatment, inorganic nutrients like phosphorus and nitrogen, fine suspended particles, heavy metals, and pathogenic microorganisms are removed (Prabu et al. 2011). It can be done through rapid sand filtration (RSF), post-precipitation, reverse osmosis, chemical oxidation, carbon adsorption, ultrafiltration, microfiltration, and dissolved air flotation (DAF) (Hamoda et al. 2002; Jolis et al. 1996; Nieuwstad et al. 1988; Ødegaard 2001; Pinto Filho and Brandão 2001). Tertiary treatment is approximately four times costlier as compared to primary treatment (de la Noüe et al. 1992).

3.1 Types of Conventional Wastewater Treatment Methods

Wastewater is mainly treated physically, chemically, and biologically (Amoatey and Bani 2011). The type of unit operations and processes in wastewater treatment shown in Fig. 2 are described below (Economic and Social Commission for Western Asia (ESCWA) 2003)

Physical Approaches

Physical methods employ physical forces to remove contaminants from wastewater (Bhargava 2016). Suspended and settable solids, oil, and grease are removed by these physical methods. Physical unit operations commonly used are:

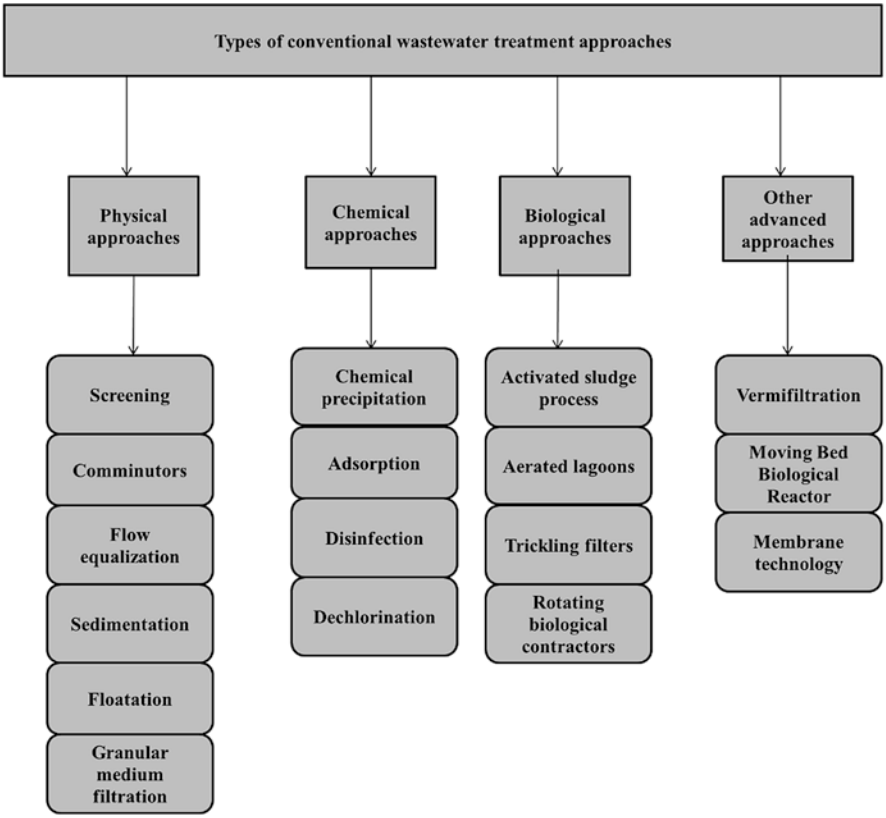

Fig. 2 Different approaches of conventional wastewater treatment. (Source: Anusha and Sham Sundar 2015; Borkar et al. 2013; Doumenq 2017; Misal and Mohite 2017; Morão 2008; Mulder 1996; Rawat et al. 2011; Shon et al. 2009)

Screening: This step employs the sieving of gross pollutants from the wastewater using devices such as parallel bars, wire mesh, rods, perforated plates, etc. After cleaning of bar screens either manually or mechanically, retained material is called screenings. This protects downstream equipment from damage (ESCWA 2003).

Comminutors: Comminutors are positioned in the middle of grit chamber and primary settling tanks and consist of rotating or oscillating cutters. These are used for reducing odors, flies, and unsightliness and for crushing the large suspended material in the wastewater flow (ESCWA 2003).

Flow equalization: Flow equalization levels out the process parameters like flow, temperature, and amount of pollutant over a period of time for ameliorating the efficacy of wastewater treatment processes like secondary and tertiary/advanced. In a wastewater treatment plant, flow equalization can be applied at many places.

Intermittent flow diversion, alternating flow diversion, completely mixed mixed flow, and completely mixed combined flow are the four basic types of flow equalization processes (ESCWA 2003).

Sedimentation: Sedimentation involves separation of suspended particles through gravity separation (WEF 2008). Particulate matter, biological flocs, and chemicals present in wastewater are eliminated in the primary settling basin, activated sludge settling basin, and chemical coagulation, respectively. Sedimentation occurring in settling tank is known as clarifier. Solid contact clarifiers and horizontal-flow and inclined-surface basins are the main designs of sludge collectors (ESCWA 2003).

Flotation: Flotation is the removal of solids or liquids from wastewater by injecting air bubbles which either attach to the liquid or get confined in suspended particles, increasing the particles' buoyant. As the particles float to the top, they can be easily removed (Koivunen 2007). Dispersed air flotation, dissolved air flotation, electroflotation (Edzwald 1995; Rubio et al. 2002), precipitate flotation, mineral flotation, and colloid flotation (Koivunen 2007) are some of the flotation techniques.

Granular medium filtration: This technique is used for the additional removal of chemically precipitated phosphorus and suspended solids from the effluent from biological and chemical treatment units. The filtration process employs two steps: filtration and cleaning/backwashing. In filtration, the waste effluent is passed to a filter bed made of granular medium with or without the addition of chemicals. Suspended materials present in wastewater are then removed by different processes like interception, adsorption, flocculation, impaction, and sedimentation. Cleaning or backwashing can be either continuous involving simultaneous filtering and cleaning operations or semicontinuous including sequential filtering and cleaning operations (ESCWA 2003).

Chemical Approaches

Chemical methods require the use of chemicals for wastewater treatment by means of chemical reactions to remove dissolved solids, nutrients, and heavy metals. Chemical unit processes are employed in synchrony with physical unit and biological unit processes (Bhargava 2016).

Chemical precipitation: In this approach, finely divided solids are flocculated into settable flocs. Coagulation-flocculation is used for the treatment of wastewater in chemical precipitation. Common coagulants used for wastewater treatment include lime ($Ca(OH)_2$), ferrous sulfate ($FeSO_4.7H_2O$), ferric chloride ($FeCl_3.6H_2O$), and alum ($Al_2(SO_4)_3.14H_2O$) (Jiménez et al. 2010). Colloidal substances responsible for the color and turbidity of the wastewater are treated through coagulation/flocculation (Arvanitoyannis and Ladas 2008). This method eliminates heavy metals and phosphorus effectively, but large amount of sludge is generated that can be dewatered and used for land filling (WEF 2008).

Adsorption with activated carbon: It involves accumulation of soluble particles present within a liquid on an appropriate interface. Activated alumina, hydroxides, activated charcoal, and resins are some of the common examples of adsorbents which are used for removal of substances like detergents and toxic compounds (Samer 2015). Activated carbon is a commonly used absorbent, and powdered activated carbon (PAC) and granular activated carbon (GAC) are its two common types (ESCWA 2003). Unlike GAC, powder activated carbon is added to wastewater using feed equipment instead of being carrying in column or bed (Corbitt 1998; Weber 1972).

Disinfection: Disinfection is the last step of wastewater treatment process for the conservation of ecosystem and human health (Sun et al. 2009). A good disinfectant should be easy to handle, inexpensive, and reliable and have potential bactericidal action (Samer 2015). Several factors affect the process of disinfection which include pH, type of disinfectant, temperature, exposure time, and type of effluent and pathogen (WEF 1996). Most commonly used disinfectants are physical agents such as light and heat, radiations (ionizing as well as nonionizing radiations), UV light, and chemical substances like chlorine and its compounds, bromine, peracetic acid (PAA), iodine, ozone, soaps and detergents, heavy metals, phenols, alcohols, etc. (Koivunen 2007; Russell 2006).

Dechlorination: For wastewater disinfection, chlorine and its derivative compounds are most commonly used, but it undergoes certain chemical reactions with the organic compounds in wastewater and produces disinfection by-products (DBPs) which have carcinogenic and mutagenic properties (Sun et al. 2009) which necessitate dechlorination (Amin et al. 2013). In dechlorination process, chlorine residues (in free and combined form) are removed from wastewater effluent (ESCWA 2003). It is done by using reducing agents such as sodium sulfite (Na_2SO_3), sulfur dioxide (SO_2), or sodium metabisulfite ($Na_2S_2O_5$) or by activated carbon (Bagchi and Kelley 1991).

Biological Approaches

Biodegradable organic matter in dissolved or colloidal form can be removed by using biological approach (Rosen et al. 1998). Contaminants are removed by the biological activity of microorganisms which degrade the organic matter in wastewater into gases (Topare et al. 2011).

Activated sludge process: Municipal wastewater is commonly treated with this process. It is an aerobic process for the elimination of BOD and suspended solids by using suspended bacterial flocs. A variety of factors which include temperature, pH, concentration of available oxygen and organic matter, waste rates, and aeration period influence the activated sludge system (Amoatey and Bani 2011). The main principle behind this process is that vigorous aeration of waste effluent generates activated sludge (flocs of bacteria) which degrades organic compounds. Activated sludge is recycled for the maintenance of concentration of active bacteria. Settling tanks are equipped with accessories like waste pumps, blowers providing aeration, and a device for measurement of flow rate. In this process, degradation occurs mainly through three main processes including microbial processes, volatilization, and sorption onto sludge flocs (Grandclement et al. 2017).

Aerated lagoons: It is a basin of about 1–4 meter depth wherein treatment of wastewater occurs either by solids recycling or flow-through basis. The aerators provide aeration, dissolved oxygen, and suspended microbial biomass for achieving maximum aerobic activity. Based on the strength and temperature of waste effluent and level of treatment, the hydraulic retention time (HRT) varies from 5 to 8 days (Samer 2015). One study reported that in household water for HRT of 5 days, 85% reduction in BOD was achieved, but BOD value decreased to 65% at 10 °C temperature (Gray 2005).

Trickling filters: A trickling filter is a basin packed with an inert carriers like volcano rock, gravels, or other synthetic material in which wastewater is supplied from the top tickles through the filter medium where organic compounds in wastewater are absorbed by microorganisms that are attached to medium as a slime layer having thickness of approximately 0.1–0.2 mm. In the outer part of slime, breakdown of organic material occurs by the aerobic microorganisms. Further, growth of anaerobic microorganisms occurs due to oxygen deprivation which makes thick layer of microbial growth. Until the microorganisms present near the surface cannot adhere to media, continuous development of biological film occurs. A section of the biological slime layer repeatedly falls off by a process called sloughing. Removal of the sloughed off portions occurs by the drain system by transferring to a clarifier (EPA 2000).

Rotating biological contractors: These consist of plastic media with diameter ranging from 2 to 4 m mounted vertically on a horizontal rotating shaft (Peavy et al. 1985). As the shaft rotates slowly with about 40% submerged media, the media coated with biomass are exposed alternately to wastewater and oxygen. Biomass oxidize the organic matter present, and excess biomass is shredded off in a downstream clarifier automatically (Amoatey and Bani 2011). These are best suited for treatment of municipal wastewater (Peavy et al. 1985). Due to their ability of quick recovery from unfavorable conditions, these have been installed in many petroleum facilities (Schultz 2005).

Other Advanced Approaches

Vermifiltration: It is a new technology that is a combination of traditional process of filtration with vermicomposting, i.e., using earthworms for wastewater bioremediation (Anusha and Sham Sundar 2015). It is a simple filtration apparatus consisting of lower layer of gravels covered with aggregates and sand layer covered with cow dung clay and a population of earthworms. As the wastewater passes through the filter bed, earthworms use fats and oils for their metabolism from it, and the leftover water percolating from bottom is collected in another vessel (Misal and Mohite 2017). The body of earthworms acts as biofiltering agent, and body wall absorbs compounds from wastewater, and reduction in wastewater COD by 80–90%, BOD_5 by over 90%, total dissolved solids (TDS) by 90–92%, and the total suspended solids (TSS) by 90–95% have been observed (Sinha et al. 2008).

Moving bed biological reactor (MBBR): A moving bed biological reactor (MBBR) is integration of activated sludge and trickling filters where biomass exists as suspended congregation of microorganisms and biofilms attached to carriers made of materials like high-density polyethylene or polypropylene (Borkar et al. 2013). The advantages of moving bed biological reactor is that it is not sensitive to load variations and other types of disturbances (Delenfort and Thulin 1997; Odegaard et al. 1994), slight head loss, and no recycling of biomass is required (Xiao et al. 2007).

Membrane technology: Membrane technology is a broad term used for different processes for transportation of substances from one phase to another phase with the aid of permeable membranes allowing passage of some specific substances while retaining others (Mulder 1996). A gradient of concentration, electric potential, temperature, and pressure acts as major driving force for solute transportation (Mulder 1996). The technology depends on physical forces, and no addition of chemicals is required (Morão 2008). Based on the driving force, membrane processes can be divided into four main types: ultrafiltration (UF), microfiltration (MF), nanofiltration (NF), and reverse osmosis (RO) (Shon et al. 2009).

3.2 Limitations of Conventional Techniques for Pathogen Removal

Though commonly used, conventional techniques are not able to remove variety of chemicals and pathogenic microorganisms from wastewater. Limitations of various conventional wastewater techniques are mentioned below:

Physical approach limitations

- Manual cleaning of different types of screen is laborious task, and overflowing may occur due to clogging. Mechanically cleaned screens operate well but jam due to obstructions (WEF 2008).
- Moreover, a substantial amount of dissolved and colloidal material is still present in waste effluent after physical treatment of wastewater (Samer 2015).

Chemical approach limitations

- Various studies have shown that physicochemical processes like coagulation and flocculation are ineffective for removing various pollutants like pharmaceuticals and endocrine disrupting compounds (EDCs) (Petrovic et al. 2003; Vieno et al. 2006; Westerhoff et al. 2005).

- Also, coagulation-flocculation generate complex sludge and are costlier (Ghoreishi and Haghighi 2003; Sirianuntapiboon et al. 2006).
- The processes of chemical unit are additive which result in net increase in the constituents of wastewater (ESCWA 2003).
- Although residual protection is provided by chlorination, against regrowth of pathogens (Szewzyk et al. 2000; Zhang and DiGiano 2002), it produces undesirable tastes and odors (Suffet et al. 1995) and forms different disinfection by-products (Becher 1999; Hozalski et al. 2001; Gopal et al. 2007; Sadiq and Rodriguez 2004). Furthermore, enteric viruses, spores of bacteria, and protozoan cysts in sewage are also not removed efficiently (Sobsey 1989).
- Chlorine and ozone are inefficacious against helminth eggs and protozoan cysts, and certain viruses like adenoviruses show high resistance against UV light (Jiménez et al. 2010).

Biological approach limitations

- The complex polluted waters consisting of pharmaceuticals, surfactants, and various industrial products cannot be treated by traditional technologies like activated sludge (Amin et al. 2014).
- Most of the contaminants remain soluble in waste effluent which cannot be removed by activated sludge and tickling filters (Servos et al. 2005; Urase and Kikuta 2005).
- The main limitations of trickling filter are having limited flexibility and problem of operation at low temperature (Metcalf and Eddy 1991; Reynolds 1982).
- Rotating biological contractors may give problem in conditions of high organic load and temperature below 13 °C (WEF 2008).

4 Suitability of Wastewater for Algal Growth and Water Quality Indicators

Microalgae are unicellular or multicellular simple structured and primordially photosynthetic organisms having a large surface-to-volume body ratio. These can thrive and grow expeditiously in severe conditions. This bestows them to take considerable proportion of nutrients from the environment where they grow. These absorb sunlight, assimilate atmospheric CO_2, and obtain nutrients from the aquatic habitat under their natural conditions. Apart from phototrophic mode of nutrition, these can be cultivated heterotrophically (i.e., utilization of organic carbon as the source of energy and carbon), mixotrophically (cultivated under both phototrophic and heterotrophic conditions), and photoheterotrophically (using light, organic carbon as carbon and energy source). Algae can be cultivated according to the availability of the resources and for the purpose to be used for (Christenson and Sims 2011). Various kinds of wastewaters can be exploited for growing microalgae, thus improving the water quality apart from reducing the demand of water and fertilizer appreciably (Prajapati et al. 2013). A number of factors are responsible for the substantial microalgal growth in wastewater. These crucial factors are temperature and pH of cultivation medium, concentration of N, P, and carbon (organic), light, CO_2, and O_2. The concentration of N and P in wastewater is higher compared to other cultivation media. Mostly the N present in it is found in the state of ammonia, and this may impede growth of algae (Konig et al. 1987; Wrigley and Toerien 1990; Pittman et al. 2011). However, it differs with the wastewater type and its treatment sites. In addition to this, the capability to sustain in different wastewater conditions varies from species to species. For example, the chlorophytic unicellular microalgal species efficiently uptake nutrients from wastewater and thus thrive in many wastewater conditions (Aslan and Kapdan 2006; Ruiz-Marin et al. 2010). Still, the efficiency of nutrient accumulation among various chlorophyte species varies. For example, Travieso et al. (1992) described that *Chlorella vulgaris* was more efficient in nutrient accumulation (N, P) from wastewater compared to *Chlorella kessleri*, and Ruiz-Marin et al. (2010) also noticed that compared to *Chlorella vulgaris*, *Scenedesmus obliquus* showed appreciable growth in municipal wastewater. In high-rate algal and oxidation ponds, the dominant phytoplanktonic communities are generally *Chlorella* and *Scenedesmus* (Masseret et al. 2000).

Microalgal species in suspension or immobilized form were found to be effective accumulators of nitrogen and phosphorus from sewage-based wastewater. Many *Scenedesmus* and *Chlorella* species can extensively eliminate (>80%) nitrate, ammonia, and total phosphorus from secondary treated wastewater (Ruiz-Marin et al. 2010; Zhang et al. 2008), thus depicting the capability of these microalgal species for sewage treatment. In case of agricultural wastewater, the N and P content is very high despite which efficient microalgal growth has been achieved in it (An et al. 2003; Wilkie and Mulbry 2002). Industrial wastewater has also been tried out for microalgal cultivation, but the algal production has been found to be less as it mostly contains high toxin concentrations (zinc, cadmium, hydrocarbons, chromium, etc.) and low phosphorus and nitrogen concentration (Ahluwalia and Goyal 2007; de-Bashan and Bashan 2010). Therefore, utilization of industrial wastewater for algal cultivation is less feasible. However, one recent study suggests potential of industrial effluent from carpet mill in furnishing nutrients for the significant algae biomass production (Chinnasamy et al. 2010). Moreover, wider availability and uniformity in composition make the agricultural wastewater and municipal more feasible for algae cultivation than the variable composition of various industrial wastewaters. Researchers have utilized various kinds of wastewater for the microalgae cultivation (Table 1).

Microalgae as Water Quality Indicators

Bioindicators consist of microorganisms or biological processes. Bioindicators assess the cumulative effect of various pollutants on water quality and how it alters with time and to what time period it may prevail. However, there is a range of indicator organisms, but algae are potential indicators for evaluating quality of water due to the following reasons:

- Easy availability of the nutrients required for growth.
- Faster growth rate.
- Shorter life cycle.
- Wider geographical distribution.

Table 1 Algal biomass production from wastewater (Rawat et al. 2016; Kumar et al. 2015; Show and Lee 2014; Rawat et al. 2011; Pittman et al. 2011)

Type of wastewater	Algae species	Biomass productivity	References
Municipal wastewater	*Chlorella* sp.	0.948 d^{-1} (growth rate)	Wang et al. (2010).
Drain wastewater	*Chroococcus* sp. 1	1.05 g L^{-1}	Prajapati et al. (2013)
Livestock wastewater	*Chroococcus* sp. 1	4.44 g L^{-1}	Prajapati et al. (2014)
Wastewater from metro plant	*Chlamydomonas reinhardtii*	2.00 g $L^{-1}d^{-1}$	Kong et al. (2013)
Urban wastewater	*Desmodesmus communis*	0.138–0.227 g $L^{-1}d^{-1}$	Samorì et al. (2013).
Piggery wastewater	*Arthrospira* sp.	11.8 g $L^{-1}d^{-1}$	Olguín et al. (2003)
Domestic wastewater with urea supplementation	*Chlorella sorokiniana*	0.2 g $L^{-1}d^{-1}$	Ramanna et al. (2014)
Piggery wastewater	*Botryococcus braunii*	–	An et al. (2003)
Sewage wastewater	*Chlorella minutissima*	0.073–0.379 g L^{-1}	Bhatnagar et al. (2010)
Carpet mill	*Scenedesmus* sp.	0.126 g $L^{-1}d^{-1}$	Chinnasamy et al. (2010)
Industrial wastewater	*Desmodesmus* sp. TAI-1 and *Chlamydomonas*	1.5–1.8 g L^{-1}	Wu et al. (2012)
Campus sewage	*Scenedesmus quadricauda*	0.052–0.082 g $L^{-1}d^{-1}$	Han et al. (2015)
Artificial wastewater	*Scenedesmus* sp.	0.996–0.119 g $L^{-1}d^{-1}$	Voltolina et al. (1999)
Anaerobically digested dairy manure	Mix of *Ulothrix zonata*, *Ulothrix aequalis*, *Microspora willeana*, *Oedogonium* sp., *Rhizoclonium hieroglyphicum*	5.5 g $L^{-1}d^{-1}$	Wilkie and Mulbry (2002)

- Bulk availability of diverse groups.
- Quick response to qualitative and quantitative changes in the environment due to pollution.
- Easier detection and sampling (Gökçe 2016).

Algae have demonstrated to be appropriate indicators of water quality. Microalgae are essential and probable bioindicators of eutrophication because of their immediate response to variations of environmental conditions resulting from the different anthropogenic activities (Kelly-Gerreyn et al. 2004; Álvarez-Góngora and Herrera-Silveira 2006; Livingston 2001). Microalgae thrive in almost all aquatic habitats besides dwelling on rocks, macroalgae, or submerged surfaces, where both planktonic and microphytobenthic assemblages are utilized for characterization of aquatic ecosystems with the use of biological, physicochemical, or hydromorphological indicators (Hermosilla Gomez 2009). Various microalgal species like *Oscillatoria*, *Chlamydomonas*, *Scenedesmus*, and *Chlorella* are used as indicators of water pollution (Padisák et al. 2006).

5 Role of Algae in Pathogen Removal

Wastewater poses many threats to the public health as it contains pathogenic microorganisms. So to attenuate this problem and to make this water usable, removal of such pathogenic microorganisms is necessary and must be a primary concern in treatment process (Jiménez et al. 2010). As there are various waterborne human pathogens (Wu et al. 2016), their assessment would be very cost-intensive. Hence, the assessment is done by monitoring of bacterial indicator organisms (like *Escherichia coli*, total coliforms, or fecal coliforms) in treated wastewater. The utilization of algae for wastewater treatment has been in trend for approximately >50 years. Oswald and Gotaas (1957) were the first to demonstrate the application of algae in treatment process. The basic principle underlying the biological treatment is to boost the removal of pathogens, nutrients, and heavy metals and to provide oxygen for the mineralization of organic pollutants by heterotrophic aerobic bacteria which ultimately leads to the production of CO_2 valuable for the agents carrying biological treatment like algae (Munoz and Guieysse 2008). The dissolved oxygen (DO) and pH of wastewater increase due to the algal activity. It has been investigated that growth of algae can facilitate the removal and inactivation of both *Escherichia coli* and total coliforms. The mechanisms and factors responsible for this have been discussed ahead in the chapter.

Removal or biotransformation of pollutants from wastewater like xenobiotics and nutrients and CO_2 from polluted air by the utilization of macroalgae or microalgae is known as phycoremediation (Mulbry et al. 2008; Moreno-Garrido 2008; Olguın 2003; Olguın et al. 2004). Microalgae either aerobically or anaerobically can treat wastewater, industrial effluents, and solid wastes through various processes. Microalgae being effective converters of solar energy can generate massive blooms and also can produce different kinds of valuable secondary metabolites (Moreno-Garrido 2008; Lebeau and Robert 2006) and are thus potential treating candidates for wastewater treatment.

6 Mechanisms Involved in Pathogen Removal by Microalgae

The various mechanisms of pathogen removal from wastewater by algae are as under:

- Competition of nutrients.
- Elevation of pH and dissolved oxygen.

- Algal toxins.
- Adhesion and sedimentation of pathogens.

Competition of Nutrients

Algae consume nutrients and carbon sources needed by the bacterial cells for their survival. This increases their retention time in water. This diminution of the sources of carbon in water may lead to the starvation of fecal bacteria due to unavailability of its energy sources, thus ultimately resulting in their death (Van der Steen et al. 2000).

Elevation of pH and Dissolved Oxygen

The photosynthetic activity of microalgae has been found to increase the pH and dissolved oxygen (DO) content in the wastewater. The elevated levels of these two factors result in the deactivation of the pathogens present in water (Muñoz and Guieysse 2006). Actually, the combined action of sunlight, pH, and oxygen through photosensitizers, in a process called photooxidation, results in the removal of pathogens from wastewater. These photosensitizers both present inside (porphyrins) and outside of the bacterial cells (dissolved organic matter) help in the absorption of light of wavelengths (400–700 nm), thereby splitting the oxygen and resulting in the formation of singlet oxygen and hydrogen peroxides, the potential agents responsible for the damage of DNA of cell membrane (Ansa et al. 2015; Curtis et al. 1992). In aquatic environments, hydrogen ion is pivotal for many metabolic reactions in microbial cells like ion transport and energy generation (Mitchell 1992). This is fundamental in major phases of water and wastewater treatment. The substantial usage of dissolved carbon dioxide by microalgae for its growth is generally responsible for the elevation of pH and DO. Algae utilize dissolved inorganic carbon through photosynthesis and liberate oxygen as a photosynthetic by-product, as given in Eq. (1).

$$6CO_2 + 12H_2O \xrightarrow{\text{light, pigment receptor}} C_6H_{12}O_6 + 6H_2O + 6O_2 \tag{1}$$

Under sufficient availability of light and nutrients, rate of removal CO_2 by algae is higher as compared to the generation of respiratory CO_2 by heterotrophic microorganisms. The resulting change in CO_2 equilibrium is illustrated in Eqs. (2, 3, and 4) (Mayes et al. 2009).

$$H_2CO_3 \leftrightarrow CO_2 + H_2O \tag{2}$$

$$HCO^-{}_3 + H_2O \leftrightarrow H_2CO_3 + OH^- \tag{3}$$

$$CO^{2-}{}_3 + H_2O \leftrightarrow HCO_{3-} + OH^- \tag{4}$$

Uptake of CO_2 from the system will shift Eqs. (2, 3, and 4) to the right to generate more CO_2 to maintain equilibrium. Due to this, pH will get increased by generation of hydroxide ions. Hence, elevated DO and pH levels are generally seen in algae-grown wastewater ponds. Warmer climate particularly daylight hours favors this type of effect (Gschlößl et al. 1998).

Algal Toxins

The microalgae like *Chlorella vulgaris* under stress and high pH have been found to produce toxins of long-chain fatty acids. These toxins have been found to be pathogen destructive in nature (Awuah 2006). A toxin called microcystin-LR produced by *Synechocystis* sp. was found to be harmful for fecal bacteria. These toxins could harm algal communities as well, but microalgae like *Scenedesmus quadricauda* and *Chlorella vulgaris* protect themselves from these toxins by producing huge amount of polysaccharides (Mohamed 2008). Also, with the elevation in the levels of chlorophyll-a, the inactivation of fecal coliform increases. The green algae remove fecal coliforms by secreting substances harmful to fecal coliforms (Ansa et al. 2012). The pathogen removal by algal toxins is still under debate. This needs the development and modification of rapid detection methods for the detection and assessment of algal toxin role in the removal of pathogens in wastewater (Litaker et al. 2008).

Adhesion and Sedimentation of Pathogens

The pathogens may attach to the solid matter that sinks as sediment and on the surface of algae (Awuah 2006). The attachment of fecal bacteria to algae in algal ponds is essential as it exposes the fecal bacteria in close proximity to the production site of severe environmental conditions like high pH and dissolved oxygen for more effect to be felt.

The rate of sedimentation is higher in aggregated bacteria compared to the planktonic form (Characklis et al. 2005). The aggregation of suspended matter is determined by the availability of polysaccharides (acid soluble) in the solution having the potential of protonation, i.e., formation of positively charged amino groups. The microalgae *Chlorella* bears a negative zeta potential or surface charge (Liu et al. 2009). Thus, these positively charged polymers neutralize the negative algal surface charge resulting into the bridging between particles. This leads to the formation of high cell density bacterial flocs which are bigger in size with quicker sedimentation rate (Henderson et al. 2008).

7 Factors Affecting Pathogen Removal by Algae

Temperature

Most microalgae species grow in the temperature range from 15 to 35 °C, and the temperatures above and below this are not favorable for microalgal growth. Because at low temperatures, rate of growth is slower, while at higher temperatures growth rate decreases due to oxidative stress. The removal efficiency was observed to have doubled on elevating the temperature from 25 to 30 °C by utilizing a symbiotic microcosm of *Chlorella sorokiniana* and a *Ralstonia basilensis* strain (Munoz et al. 2004).

pH

The photosynthetic activity apart from the algal respiration, wastewater composition, and the kind of metabolites determine the pH of the algal cultivation medium. The rise in pH during photosynthesis is due to the uptake of CO_2, and this could increase pH up to 10–11. This rise in pH could impede the activity of both bacteria and microalgae (Posadas et al. 2014). The decrease in pH by the activity of nitrifying bacteria due to the release of H^+ also decreases the removal of pathogens from wastewater (Posadas et al. 2017).

Light

Intensity of sunlight changes significantly throughout the day and the year. Light intensity of 200–400 $mEm^{-2}\ s^{-1}$ increases the algal activity (Ogbonna and Tanaka 2000). The microalgal growth and photoperiod have been found to be directly related to each other, but with high irradiance and longer photoperiod, photoinhibition and damage will occur (Molinuevo-Salces et al. 2016). Photoinhibition is prominent after noon as the flux of radiant energy per unit area can go up to 4000 $mEm^{-2}\ s^{-1}$. It is mostly observed when algal concentration is low, like during start-up (Göksan et al. 2003), because there is not enough shading from irradiance due to other microalgal cells (Contreras-Flores et al. 2003; Richmond 2000). Homogenous distribution of light in microalgal cultures is a must to obtain high biomass productivity. Microalgae grown under field conditions, for wastewater treatment, are exposed to seasonal and daily variations of irradiation which ultimately affects the microalgal waste removal potential.

Dissolved Oxygen Concentration (DOC)

Dissolved oxygen and solar irradiance are correlated to each other. As the solar irradiance increases, O_2 production also increases and vice versa. It has been illustrated that under maximal rates of photosynthesis, DOC can reach to 40 mg L^{-1}; in fact sometimes supersaturation of oxygen occurs in closed photobioreactors or on the top of open bioreactors (Posadas et al. 2015). Even oxygen concentrations above 20 mg L^{-1} have been found to be detrimental to many microalgal species, and it reduces the photosynthetic production by 98% (Matsumoto et al. 1996). The high oxygen concentration damages the microalgal cells by a process known as photo-oxidation. This damage of microalgal cells ultimately reduces the microalgal waste removal efficiency (Suh and Lee 2003).

Predators

Due to invasion by *Chytridium* sp. or any parasitic fungi, various food chain formations in the cultivation system led to unforeseen failure of process (Abeliovich and Dikbuck 1977). Microalgae in wastewater treatment process are subjected to various inhibitory products produced by other algae, phages, protozoa, bacteria, and nematodes. These can also hamper the process of removal of pathogens by microalgae (Mawdsley et al. 1995). These can be tackled by running the process for a short period of time (1 h) at low concentrations of O_2 on daily basis in order to quell the growing ability of higher aerobic organisms (Abeliovich 1986).

Operational Conditions

Apart from the abovementioned parameters, other parameters like mixing and penetration of light are of utmost importance. Mixing is the main factor as it provides proper turbulence and homogeneity in the growth medium, thus avoids the sinking of microalgal cells. It prevents the formation of gas, nutrient, and heat gradients. Mixing also leads to the shifting of microalgae from dark and light zones so the cells can perform photosynthesis actively without any problem of light saturation and light inhibition and also increases the mass transfer between the algal cells and environment, thus increasing the removal efficiency (Grobbelaar 2000; Eriksen 2008). However, mixing beyond certain frequency limit causes shear stress which has negative impact on microalgal cells.

Microalgae being photosynthetic in nature use light energy to carry out various metabolic activities like CO_2 and nutrient uptake, synthesis of biomass which actually define the wastewater treatment efficiency. Wastewater also contains various suspended particles and compounds which limit the penetration of light to the microlagal cultures. This, in turn, lowers the biomass productivity and subsequently hampers the treatment of wastewater (Markou 2015).

8 Case Studies of Removal of Several Pathogens from Wastewater by Algae

Ansa et al. (2012) evaluated varying-strength wastewater (low, medium) and a mixture of 10-day treated wastewater and raw wastewater for the effect of varying density of *Chlorella* sp. on the fecal coliform (FC) decay rate under light and dark conditions. Under dark conditions, it was found that the decay rate of FC fluctuated with chlorophyll-a concentration and for the maximum FC destruction optimum chlorophyll-a concentration was 10 ± 2 mg L^{-1}. It was further reported that under both light and dark conditions, at algal densities of ≥ 13.9 mg L^{-1}, decay rate was faster in medium-strength wastewater compared to low-strength wastewater. While under light conditions, addition of second feed of wastewater to already operating wastewater treatment process decreased the FC decay rate for varying algal densities in the range of 0.6–19.6 mg L^{-1}.

Mezzari et al. (2017) investigated the elimination of *Salmonella enterica* serovar *Typhimurium* by *Scenedesmus* sp. in swine wastewater. Photobioreactors filled with 3 L of diluted swine wastewater with and without microalgae *Scenedesmus* sp. (30% v/v, 70 mg L^{-1} dry weight) inoculated with *S. enterica* (10^5 CFU mL^{-1}) were subjected to mixotrophic cultivation using red light emission diode at 630 nm and 121.5 µmol m^{-2} s^{-1} at room temperature under continuous mixing conditions. Cell count was taken by plate count method, and qPCR amplifications of the *Salmonella* invasion gene activator, *hilA*, were executed. It was found that *S. enterica* was removed completely in the presence of microalgae within 48 h of treatment, while in the absence of microalgae, concentration of *S. enterica* increased 1.5 log CFU

mL^{-1} in 96 h. However, in photobioreactor with controlled pH *S. enterica* concentration remained constant (2.8 ± 0.2 log CFU mL^{-1}) throughout 96 h.

Ansa et al. (2011) evaluated the effect of algae *Chlorella* on pathogenic *Escherichia coli* in eutrophic lake and the significance of attachment of *E. coli* to suspended matter as well as algae. *E. coli* die-off rate in dialysis tube at different depths and locations in Weija Lake was evaluated. A significant decay of *E.coli* was reported which was attributed to increase in concentration of dissolved oxygen (DO) and pH. It was found that at chlorophyll-a concentration ≤0.08 mgL^{-1}, there exist a direct relation between chlorophyll concentration and decay rate of *E. coli*. They further reported that as concentration of chlorophyll increases with light, concentration of chlorophyll-a reaches at optimal value (0.24 mg/L) and *E. coli* decay rate decreases.

Rhizoclonium implexum (an algal species) has been reported to be efficient in the removal of coliform bacteria as well as total suspended solids, total dissolved solids, COD, BOD, total Kjeldahl nitrogen, and total phosphorus. Algal wastewater treatment is amiable in terms of its economic and environment considerations (Ahmad et al. (2014).

9 Utilization of Algal Biomass Obtained from Wastewater

Various useful products can be derived from the microalgae biomass like biofuels, bioactive compounds, etc. It can be converted to biofuels through different routes like biogas can be produced through anaerobic digestion, ethanol, acetone, and butanol by fermentation, biohydrogen by biophotolysis and dark fermentation, biodiesel through transesterification of lipids derived from it, and hydrocarbon and biocrude oils through gasification/pyrolysis (Heubeck et al. 2007).

Biogas

Microalgae can serve as an efficient fuel for biogas generation. Mixed microalgal cultures show comparable biogas quality and productivity as that of sewage sludge. Higher temperatures (55 °C) have been demonstrated to enhance biogas production (1020 L kg^{-1} VS) as compared to mesophilic range (986 L kg^{-1} VS at 35 °C) with CH_4 content ranging from 61% to 63%. At the same time, various algal species directly affect biogas production due to varied cell wall structure and composition (Mussgnug et al. 2010; Zamalloa et al. 2012). *Chlamydomonas reinhardtii* has been found to produce up to 390 L CH_4 kg^{-1} VS which is higher compared to methane obtained (100 L CH_4 kg^{-1} VS) from *Scenedesmus* lipid extraction leftovers. Cell wall structure governs the susceptibility of algal species to anaerobic digestion. Algal species such as *Arthrospira platensis*, *Chlamydomonas reinhardtii*, and *Epicrates gracilis* constitute proteinaceous cell walls lacking cellulose and hemicellulose (Mussgnug et al. 2010). The cellulose-free cell walls make these species undergo easier hydrolysis than that of carbohydrate-based cell wall (arduous to hydrolyze) species like *Scenedesmus obliquus* and *Chlorella kessleri.*

Biodiesel

Microalgae, the huge lipid reservoirs, are important renewable substrates for biodiesel production. Recently, lipids have lured the attention of scientists to alleviate the conventional fuel adversity. The lipid content is dependent on algal species, cultural conditions like nitrogen limitations, etc. (Brennan and Owende 2010). However, the condition of biomass also governs the lipid content like dried biomass of *Nannochloropsis oculata*, lyophilized biomass of *Chlorella pyrenoidosa*, algal cake of *Chlorella vulgaris* ESP-31, wet biomass of *Chlorella vulgaris* ESP-31, and dried biomass of *Chlorella pyrenoidosa* and has been observed to be 26.8, 47, 26.3, 14–63, and 56.3%, respectively (Li et al. 2011; Cao et al. 2013; Tran et al. 2013).

Bioethanol

Bioethanol production from microalgae is of substantial interest (Harun and Danquah 2011). Bioethanol production from algal biomass is less due to the limited availability of carbohydrate content (~13% dry matter) compared to rest bioethanol crops (~65% carbohydrate content of dry matter for maize) (Sheehan et al. 1998). Bioethanol can be generated from either the whole biomass or the biomass left after lipid extraction. Due to the lack of lignin, polysaccharide-rich microalgal biomass is easier to convert to fermentable sugars and then to bioethanol. The hydrolysis of starch storing microalgal species like *Chlorella vulgaris* and *Chlamydomonas reinhardtii* UTEX 90 to glucose via chemical or enzymatic processes is easy and attainable (Choi et al. 2010; Brányiková et al. 2011). Guo et al. (2013) have reported production of 0.103 g of ethanol/g of dry weight of *Scenedesmus abundans* PKUAC 12 biomass after treating with dilute acid and cellulose.

Acetone-butanol-ethanol (ABE)

There are various substrates for the production of ABE like microalgae and macroalgae (Ellis et al. 2012; Potts et al. 2012). Carbohydrate fermentation of algal biomass by saccharolytic *Clostridium* sp. leads to the production of ethanol, acetone, and butanol (Efremenko et al. 2012). Dilute acid and heat pretreated cyanobacteria resulted in the production of ethanol and butanol at concentrations of 0.29 g/L and 0.43 g/L (Efremenko et al. 2012).

Bio-oil

Bio-oil is produced from various algal species by thermo-conversion. Gasification, direct combustion, and pyrolysis are the major processes that cause thermo-conversion of algal biomass. As pyrolysis is executed out at comparatively lower temperatures than gasification and direct combustion, it is more favorable and results in the formation of products in all three states (solid, liquid, and gas) (Zhang et al. 2007). Bio-oil, the liquid product of pyrolysis, can be utilized in the transportation sector, thereby reducing the emission of greenhouse gases. The composition of bio-oil generated through pyrolysis from different microalgae species like *Chaetoceros muelleri* (Grierson et al. 2009), *Spirulina platensis* (Vardon et al. 2012), *Synechococcus* (Grierson et al. 2009), *Nannochloropsis* sp. (Borges et al. 2014), *Chlorella vulgaris* (Belotti et al. 2014; Wang et al. 2015), *Scenedesmus* sp. (Kim et al. 2014), *Dunaliella tertiolecta* (Grierson et al. 2009), *Tetraselmis chui*

(Grierson et al. 2011), and *Chlorella protothecoides* (Demirbaş 2006) has been widely studied.

Hydrogen Production
Another renewable energy source is hydrogen which has zero CO_2 emission during combustion (Nasr et al. 2013a) and produces extra energy per unit weight (Nasr et al. 2013b). It can be produced from microalgae through two biological methods, namely, biophotolysis and dark fermentation. Biophotolysis involves the utilization of light energy to generate hydrogen from water, whereas dark fermentation uses various bacteria that can ferment microalgal carbohydrates, proteins, and lipids to yield hydrogen (Das and Veziroglu 2008). *Chlamydomonas reinhardtii* has been found to be the most promising H_2 producing microalga. Table 2 presents the biohydrogen production from various microalgal species.

Feeds
High-protein feed supplements for livestock and aquaculture (Becker 1988) can be obtained substantially from algal biomass as it contains more than 50% crude protein which is manifold higher than the conventional protein sources (de la Noue and de Pauw 1988).

High-Value Products
A wide variety of high-value products like carotenoids (e.g., β-carotene), astaxanthin, long-chain polyunsaturated fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), etc. can be commercially produced by various microalgae. These are utilized as human nutritional supplements (Borowitzka 2013).

Table 2 Biohydrogen production from microalgal biomass through dark fermentation (Buitrón et al. 2017; Khetkorn et al. 2017; Roy and Das 2015; Pandey et al. 2013)

Microalgae substrate	Pretreatment applied	H_2 production	References
Scenedesmus obliquus	15 min ultrasonication at 45 °C	56 mL/g biomass	Jeon et al. (2013)
Chlorella vulgaris	1.6% HCl, 35 min	36.5 mL/g total xolids	Yun et al. (2013)
Chlorella pyrenoidosa	15 min exposure of 1% H_2SO4 at 135 °C	56.1 mL/g volatile solids	Xia et al. (2014)
Nannochloropsis oceanica	15 min exposure of 1% H_2SO4 at 140 °C	39 mL/g volatile solids	Xia et al. (2013)
Arthrospira platensis	1% H_2SO4, 140 °C microwave for 15 min, glucoamylase degradation	96.6 mL/g total solids	Cheng et al. (2012)
Cyanobacterial blooms	pH 13 for 30 min	94 mL/g volatile solids	Cai et al. (2015)
Scenedesmus obliquus	Autoclaved (121 °C, 15 min) and dried (80 °C, 16 h)	90.3 mL/g total solids	Batista et al. (2014)
Synechocystis sp. PCC 6803	Mutagenesis	190 nmol H_2 mg $chla^{-1}$ min^{-1}	Cournac et al. (2004)

10 Conclusion and Key Challenges

Conventional technologies of wastewater treatment have not proven to be enough successful in significant pathogen removal from wastewater, whereas microalgae-based wastewater treatment has shown quite a success at laboratory scale. The key challenge is to bring the technology to the field successfully. To accomplish that, robust techniques for bulk production of microalgae are required to be developed and cold weather issues need to be urgently addressed. The bigger challenge, after making the wastewater pathogen-free, is to develop cohesive wastewater treatment system, biomass generation and harvesting, and effective biomass processing to algae-based biofuels thereby utilizing all valuable components of microalgae.

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