THE CARBON FOOTPRINT HANDBOOK

Edited by

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T.V. Ramachandra and Durga Madhab Mahapatra

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1.1 THE SCIENCE OF CARBON FOOTPRINT

Carbon footprint refers to the amount of greenhouse gases (GHGs) produced due to human activities, measured in units of carbon dioxide. "Carbon footprint" assessment has gained importance in recent years and has been used as a benchmark for quantitation of GHG emissions in context of climate dynamics aided by anthropogenic, unregulated resource usage and consumption practices (Wiedmann and Minx 2007; East 2008; Finkbeiner 2009; Peters 2010). Carbon footprint (C footprint) is a subset of "ecological footprint" (Wackernagel and Rees 1996), which implies the land resources needed to immobilize/sequester the total GHG as CO_2 generated by anthropogenic activity. C footprint estimations have become more prevalent with towering GHG emissions, leading to global warming with rapid changes in the climate (East 2008). C footprint is a measure of the exclusive, total amount of CO_2 emissions that is directly and indirectly released by an activity or is accumulated over the life stages of a product (Wiedmann and Minx 2007). This is comparable to life-cycle impact assessment and is an indicator of global warming potential (GWP) and global sustainability. Although there has been a great deal of environmental deterioration, awareness on quantifying the magnitude of C footprint is scarce. However, the measurement of C footprint requires clarity on (i) inclusion of indirect emissions required for upstream production process, (ii) direct and onsite emission of the production process, (iii) accounting for all stages of the life cycle of a process in terms of services and goods, and (iv) systems boundary and quantification approaches.

The science of C footprint is frequently discussed as the well-known term carbon footprint, (Kleiner 2007) but variant terminologies to C footprint are climate footprint, GHG footprint, embodied carbon, and carbon flows (Courchene and Allan 2008; Edgar and Peters 2009). The term "carbon footprint" comprised of two words, the first of which, carbon, is the lifeline of all living organisms and constitutes the most dominant biopolymer in every single living organism. On an average, living organisms comprise of ~40–50% of C on a dry weight basis. Carbon forms the most abundant biopolymers globally and comprises the bulk of any living organic matter. For every organism, food mostly consists of C in some form; for example, the food source of human beings comes from plants and animals. The plants capture/immobilize CO_2 from the atmosphere using solar energy to combine it with water in order to create sugars from which they build their cytoskeleton (photosynthesis).

The term "footprint" explains the measurement or the impression of C and is represented as global hectares (Brown et al. 2009). However, the C footprint can have a variety of quantifying scales as to determine the impact or the stress/quantity of C emission (tons)/quantity normalized over CO_2 as CO_2 equivalents (tons of CO_2-eq.) as in the case of GHG potential/area for land measurements. For wastewater sector, it is attributed to the sum of all emissions associated with the collection, treatment, and ultimate disposal of wastewater. Significant sources of these emissions include the indirect emissions from the purchase of electricity, direct emissions resulting from the treatment process, fugitive emissions from the waste itself, and transportation-related emissions.

1.1.1 Importance and Need for Assessment

The last two centuries have witnessed a soaring increase in atmospheric concentrations of GHGs. Human activities such as industry, agriculture, deforestation, waste disposal, and, especially, unprecedented use of fossil fuel have been producing increasing amounts of GHGs. For example, the concentrations of CO_2 increased from approximately 280 parts per million by volume (ppmv) in preindustrial age to 372 ppmv in 2001 and have continued to increase by about 0.5% per year (IPCC 2007), whereas current CH_4 atmospheric concentration is going up at a rate of 0.02 ppmv per year. Similarly, there has been a substantial increase in sources of N_2O by anthropogenic activities of about 40%–50% over preindustrial levels. Owing to this rapid increase in GHG concentrations in the atmosphere, due to human-induced activities, the entire humanity is under the threat of global warming (rise in global temperature) and associated climate change and its far-reaching consequences.

The magnitude of impact of GHGs differs and is based on the radiative forcing together with the mean time span of the existence of GHG gas in the atmosphere, which is jointly expressed as the GWP and mostly expressed as carbon dioxide equivalent (CO_2-eq). The most significant contributors to global warming are the six Kyoto gases and, to a lesser extent, the chlorofluoro carbons (highlighted during the Montreal protocol; IPCC 2006, 2007). The largest share of the GHG is contributed by CO_2 (~60%) followed by methane and nitrous oxide and the largest GHG-producing sector being the energy supply (~26%) (IPCC 2007). The global anthropogenic GHG emissions and the GHG emissions by various sectors (IPCC 2007) have been elucidated in Figure 1.1.

Anthropogenic GHG emissions grew at an average annual rate of 2.2% during 2000–2010, compared with a growth rate of 1.3% per year in the period 1970–2000. CO_2 emissions from fossil fuels and industrial processes contributed 65% of global GHG emissions in 2010. The increase in anthropogenic GHG emissions comes from energy supply (47%), industry (30%), transport (11%), and building (3%) sectors. Globally, population and economic growth continue to be the most important drivers of increase in CO_2 emissions from fossil-fuel combustion (IPCC 2014).

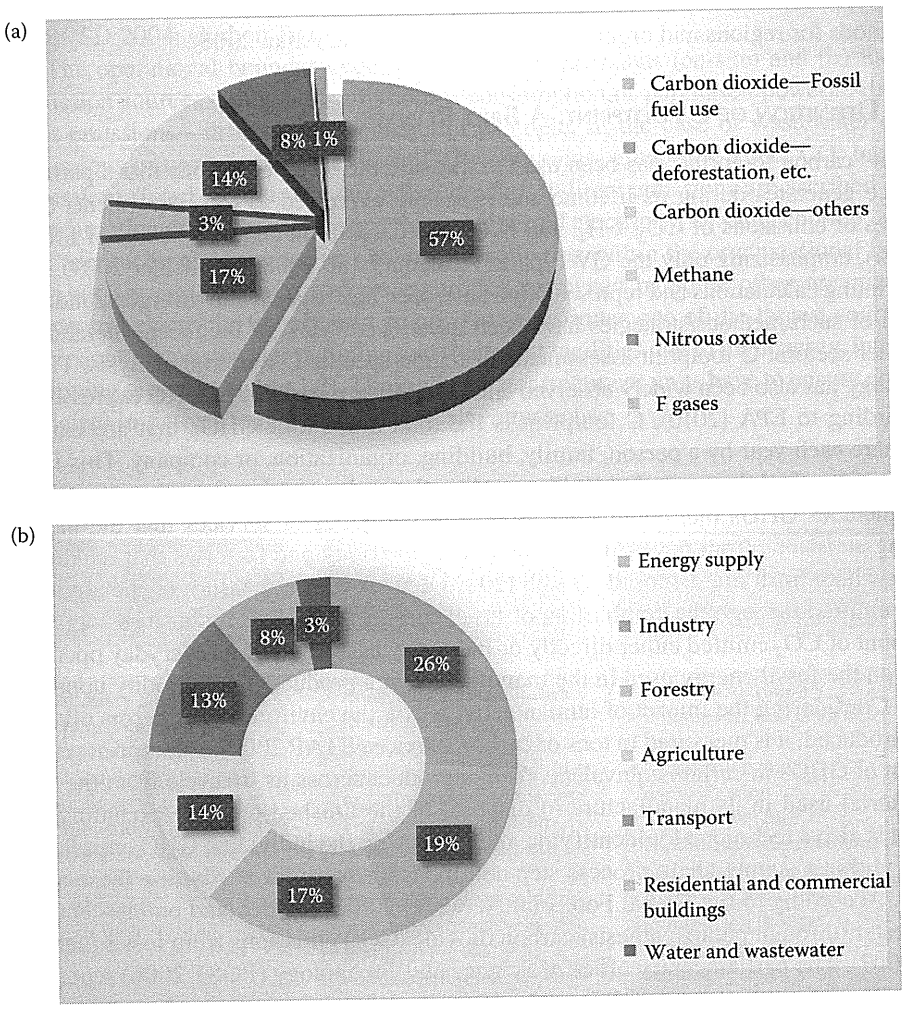

FIGURE 1.1 (a) Global anthropogenic GHG emissions. (b) GHG emissions by sector. (From IPCC. 2007. Climate change 2007: Synthesis report: Contribution of working groups I, II and III to the fourth assessment report. Intergovernmental Panel on Climate Change (IPCC).)

The greenhouse effect of these gases is estimated by the GWP that depends on radiative forcing and the timeframe of consideration (usually taken as 100 years). The GWP factors for a 100-year horizon for (a) CO_2 is 1 (base/reference), (b) CH_4 is 21, and (c) N_2O is 298 (IPCC 2007). In other words, over a time period of 100 years, 1 ton of CH_4 will have a warming effect equivalent to 25 tons of CO_2 (IPCC 2006).

Increase in the emission levels has led to 0.74°C rise in the mean global temperature (IPCC 2007), which in turn can lead to global warming resulting in glaciers melting followed by increase in sea level and submersion of coastal and low-lying areas (Kerr 2007). The imbalance in nature is evident through the recent episodes of cyclones, tsunamis, and extreme weather conditions. The C footprint is accounted as a quantitative expression of the GHG emissions (Carbon Trust 2006) from various activities that aid in devising tools/methods for emission management and figuring out appropriate mitigation measures. Today, it has become imperative for a detailed GHG inventory on a global scale, and specific methods have to be devised for C abatement to reduce and evaluate C footprint and GHG emission. The C footprinting exercise will enable quantification of emissions, tracing important sources of emissions for which appropriate management and emission reduction techniques can be developed along with increasing the efficiency of the process. Stringent legislative measures should be followed for accounting C footprint and identification of C footprint reduction methods for regions and organizations (Courchene and Allan 2008).

1.1.2 Definition of C Footprint: A Brief Review

The term "carbon footprint" has been used to measure the GHG emissions that a particular product or service generates during its lifetime and is mainly expressed as CO_2 equivalents (CO_2-eq.) that comprises of emissions of CO_2, CH_4, and N_2O. The C footprint can be a subset of life-cycle analysis (LCA), emphasizing only the GWP part (Weidema et al. 2008; Finkbeiner 2009). However, the C footprinting calculations and representation have been inconsistent and divergent, and the utility and potential of such methods/strategies have been questioned. Most of the recent literatures have dealt with either sectoral C footprint assessments or region-specific C footprint analysis. The C footprint terminology has also been widely observed and reported as a measure of GHG expressed as CO_2-eq.

According to EPA (2010), C footprint is the total amount of GHGs that are emitted into the atmosphere each year by a person, family, building, organization, or company. This includes GHG emissions from fuel that an individual burns directly, such as by heating a home or riding in a car. It also includes GHGs that come from producing the goods or services that the individual uses, including emissions from power plants that make electricity and factories that make products and landfills where trash are disposed. Grubb (2007) defines it as a measure of the amount of carbon dioxide emitted through the combustion of fossil fuels. In the case of a business organization, it is the amount of CO_2 emitted either directly or indirectly as a result of its everyday operations. It also reflects on the fossil energy used in the manufacture of a product or commodity upon reaching the market. Considering the impact of human activities on the environment in terms of the amount of GHGs produced, it is measured in tons of carbon dioxide (ETAP 2007). C footprint reflects the total emission of GHGs in carbon equivalents from a product across its lifecycle from the production of raw material used in its manufacture to disposal of the finished product (excluding in-use emissions). It is also a technique for identifying and measuring the individual GHG emissions from each activity within a supply-chain process step and the framework for attributing these to each output product. According to the Global Footprint Network, 2007, "the demand on bio-capacity required to sequester (through photosynthesis) carbon dioxide (CO_2) emissions from fossil fuel combustion" (GFN 2007). The parliamentary office of Science and Technology (POST 2006) reports "A 'carbon footprint' is the total amount of CO_2 and other greenhouse gases, emitted over the full life cycle of a process or product. It is expressed as grams of CO_2 equivalent per kilowatt hour of generation (gCO_2-eq./kW h), which accounts for the different global warming effects of other greenhouse gases."

1.1.3 Issues Related to Quantification: Methodological Issues

i. Greenhouse gas records and measurements

a. *GHGs*: The choice of the GHGs depends on the nature, requirement, and the guidelines of the sector/activities. For example, in the case of wastewater, the most important gas emitted is CO_2 as an outcome of bacterial metabolism, and along with it are the emissions of CH_4 and N_2O. In the case of any industry, for example, coal-based power plant CO_2 becomes the most important GHG compared to CH_4 and other gases found in traces. Many earlier studies account only CO_2 emissions for measurements of C footprint (Wiedmann and Minx 2007; Craeynest and Streatfeild 2008) and six major GHG emissions (Bokowski et al. 2007; Energetics 2007; Garg and Dornfeld 2008; Matthews et al. 2008a,b; Peters 2010).

b. *Systems boundary*: The systems boundary delineates the targeted region and considers the activities within the boundary. This depends on the organizational and operational boundaries. The organizational boundary can be ascribed as the boundary of the organization on the economic and business grounds and its associated activities under the study. The operational boundary involves the direct and indirect emissions (WRI/WBCSD 2004; Carbon Trust 2007a,b; BSI 2008).

The operational boundary considers (i) direct emissions (on-site) and (ii) indirect emission sources (embodied emissions—consumption of purchased power). All indirect emissions—difficult to quantify, for example, in the case of wastewater direct emissions, sources include all direct GHG emissions, except the direct CO_2 biogenic emissions (fixed biologically during treatment). Indirect emissions are ascribed to the consumption of purchased energy, that is, acquired electricity, heating, cooling, and so on, and such emissions are an outcome of activities within the organizational boundary but are emitted from sources controlled or owned by some other organization, as central electricity supply, and so on. This constitutes one of the largest sources of emissions for wastewater pumping and treatment facilities due to highenergy, intensive pumping process (Figure 1.2). Thus, wastewater treatment units have to ensure energy-efficient initiatives to reduce indirect GHG emissions.

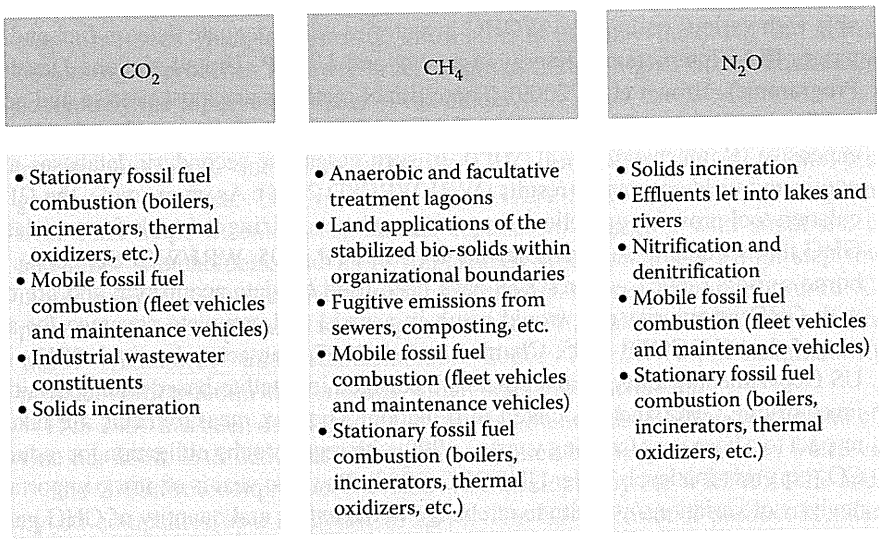

FIGURE 1.2 Method of accounting indirect emissions.

Other indirect emission sources are:

a. Supply-chain GHG emissions as upstream/downstream transport of chemicals, materials, and fuels and upstream production
b. Bio-solids reuse, including land application or other methods that are outside the organizational boundary
c. Landfilling of bio-solids
d. Emissions from services contracted with outside vendors as bio-solids hauling fuel emissions or contracted wastewater treatment by adjacent municipalities
e. Emissions from employee commuting and business travel

All these are difficult to quantify and require additional guidelines for facilitating inventories. Whatever C footprint studies conducted to date have emphasized and accounted for the quantity of GHG removal, but gross sequestration of GHGs has not been addressed (Peters 2010). Therefore, C sequestration becomes vital in all such calculations and is to be incorporated in C footprint measurements.

c. *Compilation of GHG emission data*: Emissions have been quantified either directly through on-site real-time measurements or through estimations based on the emission factors and various empirical models. The emission factors or the model-based data generation are the most used means for acquiring data. These emissions are calculated from specific emission factors using the data on fuel consumption, efficiency of the process, energy, and other activities resulting in emissions (CO_2, in particular). Emission factors for various sectors and industrial processes and land-uses are available in PAS 2050 (IPCC 2006, 2007). Country-specific emission factors developed in many countries as in national laboratories under UNFCC, US EPA (WRI/WBCSD 2004; IPCC 2007). However, the verification of these is highly required at varied organizational, operational, and geographical contexts. The region-specific emission factors and models have been precisely developed (WRI/WBCSD 2004; IPCC 2006). In cases of fugitive emissions, the direct measurements are done through specialized GHG-capture techniques such as optical, biochemical, IR sensors, and quantitative measurements through IR spectroscopy and gas chromatography (USCCTP 2005; Berg et al. 2006). Many other advanced techniques include flux towers (Velasco et al. 2005) and cavity ring-down spectrometers for measurements in air (Kelly et al. 2009). Apart from the primary data collection techniques, a wide variety of secondary data sources are accessible at the global scale in recent times. On a much larger scale, a CO_2 emission database from different countries is now obtainable with various national-level GHG inventories with adequate data on fuel and energy usage, IEA (International Energy Agency), and UNDP (United Nations Development Programme) (Brown et al. 2009). These direct methods are most precise and accurate, but the cost incurred for the data acquisition through experiments and analyses are quite expensive (Ramachandra et al. 2013). In such cases, the secondary databases are economic and yield acceptable results (WRI/WBCSD 2004). As an example, the GHG calculation tool provides guidelines for setting and customizing the tools for calculating the GHG flux specific to sector/organization (USCCTP 2005; WRI/WBCSD 2006). Spaceborne sensors (optical, microwave) have been used for data acquisition and are coupled with GHG inventories that would result in a vivid and complete coverage for acquisition of data (USCCTP 2005; Chambers et al. 2007; Ramachandra et al. 2013). NASA, US Department of Energy, and others are engaged in satellite-based data acquisition and inventorying GHG and associated information. The flux measurements are taken with respect to a base year (starting year) as 1990 with regard to the obligation for reduction of CO_2-eq. emission levels under UNFCCC (2008). The base year is of prime importance for analysis of variations/magnitude of change in the extent and quantity of GHG gases and often needs to have reproducibility and reliability (Carbon Trust 2007b; Brewer 2008a,b).

d. *Calculation of C footprint*: This is done for all GHG gas quantities/flux as CO_2-eq. considering emission factors (WRI/WBCSD 2004; Wiedmann and Minx 2007; BSI 2008). There are two approaches followed for quantification of C footprint: (a) bottom-up, based on process analysis (PA), and (b) top-down, considering environment input–output analysis. The bottom-up approach inherits the understanding of environmental impacts of individual product/region/category from the source to the sink. This approach has disadvantage for identification of appropriate system boundary and is based only on-site and most first-order impacts (Lenzen 2001). This approach becomes far fetching with increase in the systems area such as sectors/governments, and so on. In contrast, the top-down or the environmental input–output (EIO) analysis (Wiedmann et al. 2006) is most commonly applied in economics and provides a majority of the economic activities at sectoral scales. This approach can result in a robust economic set up as the boundary. Input–output approach is largely carried out for sectoral C footprinting assessment and might not be suitable for small operations/processes. Hybrid approach integrates PA with the EIO approach (Suh et al. 2004; Heijungs and Suh 2006).

e. *C footprints assessment of a product*: C footprint assessment of a product is done either through bottom-up, based on PA, or top-down, based on EIO analysis considering the full life-cycle impacts through LCA, (Life Cycle Assessment).

   Bottom-up method involves a PA considering the environmental impacts of individual products from cradle to grave. However, this will have a problem of a system boundary only on-site, that emphasizes the need for identification of appropriate system boundaries. PA-based LCAs run into difficulties for larger entities such as government, households, or particular industrial sectors.

   Top-down approach involving EIO analysis provides a picture of all economic activities at the sector level. This with consistent environmental data aids in a comprehensive and robust way taking into account all impacts of the whole economic system as boundary. Application of this approach to assess microsystems such as products or processes is limited, as it assumes homogeneity of prices, outputs, and their carbon emissions at the sector level. Advantage of input–output-based approaches is a much smaller requirement of time and manpower once the model is in place.

   The best option for a comprehensive and robust analysis is to combine PA and input–output methods. Such an approach allows one to preserve the detail and accuracy of bottom-up approaches in lower-order stages, whereas higher-order requirements are covered by the input–output part of the model.

f. *C footprint standards for products*: Carbon footprint of a product involves measuring, managing, and communicating GHG emissions related to the product's goods and services. This is based on an LCA from a global warming perspective. This helps in enhancing market reputation, engaging with suppliers, clients, and other stakeholders, and more importantly this constitutes a first step toward a more comprehensive environmental assessment. Three commonly used C footprint standards applicable to the product are: PAS 2050, GHG Protocol, and ISO 14067 (Kulkarni and Ramachandra 2009; http://www.pre-sustainability.com/product-carbon-footprint-standards-which-standard-to-choose).

   PAS 2050 has been developed by the British Standards (BSI) and came into effect in October 2008. PAS 2050 has already been applied by many companies worldwide.

   The GHG Protocol product standard was developed by the WRI/WBCSD and tested by 60 companies in a road test in 2010. The GHG Protocol product standard was launched in October 2011.

ISO DS 14067 specifies principles, requirements, and guidelines for the quantification and communication of the C footprint of a product (CFP), based on International Standards on LCA (ISO 14040 and ISO 14044) for quantification and on environmental labels and declarations (ISO 14020, ISO 14024, and ISO 14025).

All three standards developed on existing LCA methods established through ISO 14040 and ISO 14044 provide requirements and guidelines on the decisions to be made when conducting a C footprint study. Decisions involve LCA issues, such as goal and scope definition, data collection strategies, and reporting. Moreover, these standards provide requirements on specific issues relevant for C footprints, including land-use change, carbon uptake, biogenic carbon emissions, soil carbon change, and green electricity.

1.1.4 GHG Emissions from Wastewater Sector

GHGs produced during wastewater treatment are CO_2, CH_4, and N_2O. Globally, wastewater is the fifth largest emitter of CH_4 and sixth largest emitter of N_2O that contributes ~10% of the total CH_4 emissions and 3% of N_2O emissions, respectively. CH_4 and N_2O emissions are expected to grow by ~20% and 13% by 2020 (US EPA 2006). GHGs such as CO_2 are emitted due to (a) treatment process and (b) electricity consumption (Ramachandra et al. 2014a). The next section discusses GHGs due to wastewater treatment based on field experiments. Bengaluru city generates ~1200 million liters per day (MLD) of domestic wastewater that gets either partially treated or untreated and joins receiving surface waters at various locations in the city. This has contributed toward C footprint.

The GHG emissions of the select treatment plants were estimated based on conventional treatment technologies and the energy units consumed during the operation of the treatment plants. The direct GHG emissions accounted for CO_2 generated through organic matter degradation (aerated tanks-aerobic process), CH_4 generated from the clarifiers and also from the basins, N_2O emissions from the effluent discharge into surface water bodies and the diesel generator used in the treatment units. Indirect emissions were calculated from offsite generation of electric power that is consumed at wastewater treatment plant, which is explained in Section 1.2.

Wastewaters generated from domestic households are a source of nutrients contributing to growth and development of algae and consequent lipid extraction. The CO_2 sequestration in algae is accomplished by (a) direct sequestration, which comprises the capture of CO_2 from wastewaters before its emission to atmosphere and subsequent storage and (ii) indirect sequestration, based on the capture of CO_2 that is already in the atmosphere, through photosynthesis. Carbon present in wastewater can be either inorganic C (carbonic acid/bicarbonates/carbonates) or organic C (dissolved organic acids, etc.). Many wastewater algae capable of consuming both C forms are called mixotrophic algae and are extremely beneficial in rapid C fixation and reduction of wastewater C footprint. Often, the bulk of the wastewater C fixed in the algal biomass is stored in the form of lipids (neutral) that can be potentially used as feedstock for biofuel as biodiesel and gasoline. The sequestration aspect of microalgae is discussed in Section 1.3.

Increased and unprecedented population growth has resulted in enormous stress on potable water from a daily consumption point of view and also with regard to increased wastewater generated by the city. Land-use analyses show 584% growth in built-up area during the last four decades with the decline of vegetation by 66% and water bodies by 74%. Apart from these, rapid urbanization in recent times has led to mammoth wastewater generation. Untreated or partially treated wastewaters are fed to surface water, leading to GHG emissions. The sustained inflow of untreated or partially treated sewage to wetlands leads to the enrichment of nutrients such as carbon (C), nitrogen (N), and so on. Installation of conventional treatment plants has helped in the removal of dissolved and suspended biological matter, while the effluents are still nutrient-rich and need further treatment and stabilization (Ramachandra et al. 2014a,b).

Section 1.4 presents the possibility of mitigating GHG emissions from wastewater sector through integrated wetlands ecosystem. Integration with wetlands (consisting of typha beds and algal pond) would help in the complete removal of nutrients in a cost-effective way. However, this requires regular maintenance of harvesting macrophytes and algae (from algal ponds). Harvested algae would have energy value, which could be used for biofuel production. The joint activity of algae and macrophytes in the wetland systems helps in the removal of 77% chemical oxygen demand (COD), ~90% biochemical oxygen demand (BOD), ~33% NO_3—N, and ~75% PO_4^{3-}–P. Implementation of integrated wetland system helps in treating the water and more importantly in the mitigation of GHG emissions from wastewater sector.

1.2 C FOOTPRINT OF MUNICIPAL WASTEWATER

Indirect emissions due to electricity consumption (fossil fuel-based energy: coal, gas, oil) in the treatment plants have been considered along with GHG production during the wastewater treatment process. Coal-based power generation has been considered, which is the most common in India. The GHG emitted from treatment plants depends on the technology. The wastewater treated in the various treatment plants in the city are also sources of CH_4. N_2O is formed during the denitrification processes, while CO_2 results from both aerobic and anaerobic processes. GHGs such as CO_2 are emitted due to (a) treatment process and (b) electricity consumption. During an anaerobic process, the BOD of wastewater is either incorporated into biomass or is transformed into CO_2 and CH_4. A small quantity of this biomass is further converted into CO_2 and CH_4 via endogenous respiration. Other emission sources of carbon dioxide are sludge digesters (if operational). But, during aerobic treatment, CO_2 is produced through the breakdown of organic matter in the activated sludge process and some through the primary clarifiers. CH_4 can be produced from wastewater directly and/or from wastewater sludge during anaerobic digestion. The magnitude of methane emission depends on the nature of environment (redox potential), temperature, and more importantly on the amount of degradable organic matter in wastewater. N_2O gas emanates as a decomposable product of nitrate, urea, and proteins. Centralized wastewater treatment systems comprise a variety of unit processes, for example, ponds, lagoons, and advanced treatment technology that might lead to N removal and discharge to receiving waters. Both nitrification and denitrification occur, to some extent, during the treatment process. N_2O is an intermediate product of both processes and is more often associated with denitrification.

1.2.1 GHG Emissions for Wastewater Treatment Plant at Bengaluru

Bengaluru city generates ~1200 MLD of domestic wastewater that gets either partially treated or untreated and joins receiving surface waters at various locations in the city. The locations of the treatment plants are depicted in Figure 1.3 and the details regarding the installed capacity and the technology used are provided in Table 1.1. Most of the treatment plants follow an extended aeration or activated sludge process. The estimation of C has been performed based on a general mechanically aerated process set up as shown in Figure 1.4.

Methods: The quantification of C footprint has been done through field experiments (during 2012–2013) and also through GHG protocol (as per IPCC 1996; 2006). Preparation of GHG inventory involves:

a. *Organizational boundaries:* This includes the wastewater treatment plants and the electricity supply to run the treatment plants.
b. *Operational boundaries:* This deals with calculation of emissions associated with the operation/working of the treatment plants.

Measuring direct GHG emissions: Here CO_2, CH_4, and N_2O concentrations are quantified for the operational wastewater treatment plant. CO_2 emissions as an outcome of

FIGURE 1.3 Greater Bengaluru and the locations of the existing wastewater treatment plants.

TABLE 1.1
Wastewater Treatment Plants with Their Installed Capacities and the Various Treatment Technologies Used in Bengaluru

S. No.	Location	Capacity in MLD	Treatment Facility
		Bengaluru—CWSS I, II, III Stages	
1	Vrishabhavati Valley	180	Secondary: trickling filters
2	K & C Valley I	163	Secondary: activated sludge process
3	Hebbal Valley	60	Secondary: activated sludge process
4	Madivala	4	Secondary: UASB + oxidation ponds + constructed wetlands
5	Kempambudhi	1	Secondary: extended aeration
6	Yelahanka	10	Activated sludge process + filtration + chlorination tertiary
		Bengaluru—CWSS IV Stage, Phase I	
7	Mylasandra	75	Secondary: extended aeration
8	Nagasandra	20	Secondary: extended aeration
9	Jakkur	10	Secondary: UASB + extended aeration
10	K.R. Puram	20	Secondary: UASB + extended aeration
11	Kadabeesananahalli	50	Secondary: extended aeration
12	K & C Valley II	30	Secondary: extended aeration
13	K & C Valley III	55	Secondary: CMAS
14	Rajacanal	40	Secondary: extended aeration
	Total	**718**	

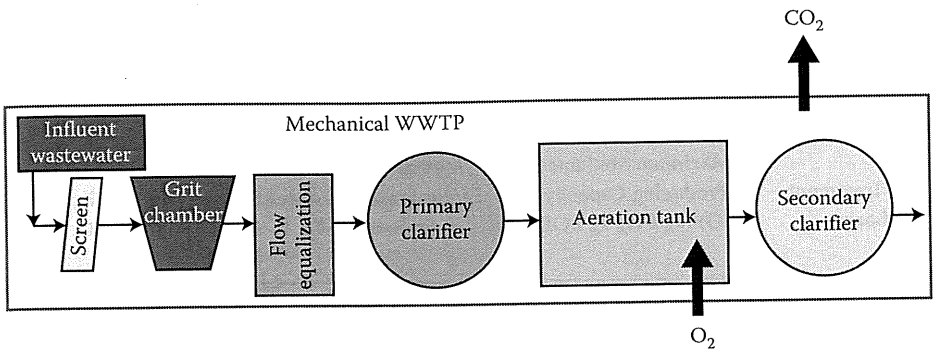

FIGURE 1.4 A schematic representation of a mechanical wastewater treatment plant.

microbial C transformation during treatment have not been taken into account based on IPCC Guidelines as they are biogenic and not from fossil sources.

Indirect GHG emissions: Here emissions occurring due to import of electricity and steam/gas are accounted and taken into consideration.

c. Estimation of emissions over time: The GHG emissions have been calculated for 12 months (to account for seasonal variations, during 2012–2013).
d. Quantification of GHG emissions: This was performed according to IPCC Guidelines for National Greenhouse Gas Inventories (1996; 2006) for quantification of GHG emissions from wastewater treatment plants.

1.2.2 Quantification of GHG Emissions in Treatment Plants

The GHG emissions for the various treatment plants were estimated based on conventional treatment technologies and the energy units consumed during the operation of the treatment plants considering the volume of water (functional unit). The direct GHG emissions were accounted for CO_2 generated through organic matter degradation (aerated tanks-aerobic process), CH_4 generated from the clarifiers and also from the basins, and N_2O emissions from the effluent discharge into surface water bodies and the diesel generator used in the treatment units. Indirect emissions were calculated from offsite generation of electric power that is consumed at wastewater treatment plant.

The estimation of the wastewater C footprint of the present wastewater treatment plants in Bengaluru is presented in Tables 1.2 through 1.9. The results show the highest C footprint through emissions by electricity usage, that is, ~38.4 ktons CO_2-eq. The total emission is ~41.53 ktons CO_2-eq., CH_4 being produced in treatment plants should be captured and used for cogeneration of electricity or can be used as a fuel on site. Although magnitude of N_2O generated was

TABLE 1.2
Estimation of Organically Degradable Material in Domestic Wastewater

	I	II	III	IV
City	Population (P)	Degradable Organic Component (BOD) (kg BOD/cap/year)	Correction Factor for Industrial BOD Discharged into Sewers	Organically Degradable Material in Wastewater (TOW) (kg BOD/year) IV = I × II × III
Bengaluru	8,499,399	12.41	1.25	132,803,109.4

TABLE 1.3
Estimation of Methane Emission Factor for Domestic Wastewater

	I	II	III
Type of Treatment or Discharge	Maximum Methane-Producing Capacity (BO) (kg CH_4/kg BOD)	Methane Correction Factor for the Treatment System (MCF_j)	Emission Factor (EF_j) (kg CH_4/kg BOD) III = I × II
Aerobic	0.6	0.05	0.03

TABLE 1.4
Estimation of Methane Emissions from Domestic Wastewater

	I	II	III	IV	V	VI	VII	VIII	IX	X
Income Group (High, Medium, and Low)	Fraction of Population Income Group (U_i) Fraction	Degree of Utilization (T_{ij}) Fraction	Emission Factor (EF_j) (kg CH_4/kg BOD)	Organically Degradable Material in Wastewater (TOW) (kg BOD/year)	Sludge Removed (S) (kg BOD/year)	Methane Recovered and Flared (R) (kg CH_4/year)	Net Methane Emissions (CH_4) (kg CH_4/year) VII = [(I × II × III) × (IV − V)] − VI	GWP for CH_4 (IPCC 2007)	Total CO_2-eq. kg CO_2-eq./year	Total CO_2-eq. tCO_2-eq./year
HI	0.3	0.07	0.03	132,803,109.4	0	0				
MI	0.5	0.03								
LI	0.2	0.1								
Avg.	0.333	0.066								
Total							86,774	25	2,168,350	2168.35

TABLE 1.5
Estimation of Nitrogen in Effluent

I	II	III	IV	V	VI	VII
Population (P)	Per Capita Protein Consumption (Protein) kg/person/year	Fraction of Nitrogen in Protein (Fnpr) kg N/kg protein	Fraction of Nonconsumption Protein (Fnon-con)	Fraction of Industrial and Commercial Codischarged Protein (Find-com)	Nitrogen Removed with Sludge (Default is Zero) (N sludge) kg	Total Nitrogen in Effluent (N effluent) VII = [(I × II × III × IV × V)] − VI
8,499,399	0.056	0.16	1.4	1.25	0	133,270.58

TABLE 1.6
Estimation of Emission Factor and Emissions of Indirect N_2O Emissions from Wastewater

I	II	III	IV	V	VI	VII	VIII
Nitrogen in Effluent (kg N/year)	Emission Factor (kg N_2O-N/kg N)	Conversion Factor of kg N_2O-N into kg N_2O 44/28[a]	Emissions from Wastewater Plants (Default as Zero) kg N_2O/year	Total N_2O Emissions kg N_2O/year	GWP for N_2O (IPCC 2007)	Total CO_2-eq. kg CO_2-eq./ year	Total CO_2-eq. tCO_2-eq./year
133,271	0.0005	1.57	0	104.62	298	31,176.76	31.18

[a] Indicates the division of the molar mass of the gas i.e. N_2O by the amount of di-nitriogen. This is found to be [44 (MM of N_2O)/28 (MM of N2) = 1.57].

TABLE 1.7
Emissions from Diesel Generator Set during Treatment Process

Total Diesel Consumption (L/year)	Emission Factor (tCO_2-eq./L) of Diesel	Total CO_2-eq. (tCO_2-eq./year)
364,000	0.00255	928.2

TABLE 1.8
Electricity Consumption[a] of the Plant

Total Electricity (MWH)	Emission Factor (0.91 tCO_2-eq.)	Total Scope 2 Emissions CO_2-eq. (tCO_2eq./year)
42,200	0.91 tCO_2/MwH	38,402 tCO_2-eq.

[a] The electricity consumption includes the power required for (a) pump station, (b) clarifiers, (c) aerators, (d) control room, and (e) sludge pump.

TABLE 1.9
Total Emissions of the Treatment Plants[a]

Emission Scopes	Source	CO_2-eq. Emissions
Scope I	CH_4	2168.35 tCO_2-eq.
	N_2O	31.18 tCO_2-eq.
	Generator (fuel)	928.2 tCO_2-eq.
Scope II	Electricity used	38,402 tCO_2-eq.
Scope III	Not attempted	
Total		**41,529.73 tCO_2-eq.**

[a] Biogenic CO_2—36,135 tCO_2 per year.

comparatively much less than other components due to low nitrification, the CO_2 generated during the treatment process is huge. Considering the entire wastewater volume of Bengaluru region as 1200 MLD, the total organic carbon (TOC) in city wastewaters is about 180 tons/day. Therefore, the net CO_2 generated in the system is about 99 tons/day. This accounts to ~36,135 tCO_2 per annum. Considering the biogenic CO_2 for the footprinting, the total C footprint of Bengaluru region is ~77,665 tCO_2-eq./year.

1.3 CARBON SEQUESTRATION AND BIOFUEL PROSPECTS

Wastewater treatment is still a challenge in India due to the enormous load (1,70,000 MLD). At the same time, escalating fuel prices and nonavailability of indigenous oil reserves have necessitated viable oil options for automotive transportation, energy generation, and so on. The resident algal species such as euglenoides occurring naturally in the wastewater lagoons due to their heterotrophic nature are ideal for wastewater treatment (and mitigation of carbon emissions) as well as lipid production.

The CO_2 sequestration in algae is accomplished as (a) direct sequestration, which comprises the capture of CO_2 from the wastewaters before its emission to atmosphere, and its subsequent storage and (ii) indirect sequestration, based on the capture of CO_2 that is already in the atmosphere, through photosynthesis. Carbon present in wastewater can be either inorganic C (carbonic acid/bicarbonates/carbonates) or organic C (dissolved organic acids, etc.). Many wastewater algae are capable of consuming both C forms and are called mixotrophic algae and are extremely beneficial in rapid C fixation and reduction of wastewater C footprint. Often, bulk of the wastewater C fixed in the algal biomass is stored in the form of lipids (neutral) that can be potentially used as feedstocks for biofuel as biodiesel and gasoline.

Soaring global oil demands and perishing stock of finite oil reserves have created enormous interest for a clean, green, and alternative energy sources such as algal biofuels (Ramachandra et al. 2009; Ackom 2010). Among the major organisms considered for oil, bacteria accumulate lipid up to 80% of dry weight (Gouda et al. 2008), yeast up to 70% (Li et al. 2007; Easterling et al. 2009; Hu et al. 2009), algae up to 50%–75% (Chisti 2007), and fungi up to 40% of dry weight (Seraphim et al. 2004). Among these, algae are promising due to their higher growth rates in natural conditions (sunlight and atmospheric CO_2) with minimal feed requirements (Chanakya et al. 2012; 2013). Species such as euglenoides have higher lipid generation (Coleman et al. 1988) with continuous growth and typical mixotrophic mode of nutrition (Yamane et al. 2001). Microalgae contain high amount of lipids, including triglycerides (Borowitzka 2010), suitable for production of biodiesel. Microalgae species *Botryococcus braunii* have a very high content of hydrocarbons similar to crude oil of oil deposits (Metzger and Largeau 2005). These microalgae thrive well in tropical climates in any biosystems like open ponds, eutrophic lakes, wastewater lagoons, algal ponds, and so on. Wastewaters generated from domestic households are a source of nutrients contributing to growth and development of algae and consequent lipid extraction. Lagoons are also cost-effective wastewater treatment options adopted in most part of the country, paving the path for a sustained energy generation.

Neochloris oleoabundans (Li et al. 2008), *Chlorella protothecoides* (Xu et al. 2006), and *Chlorella vulgaris* (Liang et al. 2009; Widjaja et al. 2009) are some reported high lipids accumulating strains in controlled conditions. However, studies on the resident algal population/species in natural environments are scarce. In this context, investigations on euglenoid *Lepocinclis ovum* focusing on the growth environments, cell characteristics in relation to the lipid content, composition of lipid, and quantum of lipid production have been done for the first time. Growth and consequent oil accumulation under natural conditions deviate significantly from that of photobioreactors with the favorable experimental conditions. In the natural conditions, *L. ovum* competes for dominance with other algal species and predators under hostile conditions. Algae under such stressed conditions appear to have higher lipid accumulation and prove to be a viable candidate for biofuel generation. Generation of a large quantity of wastewater and subsequent treatment through lagoons (for economic reasons) provide an opportunity to harvest microalgae for biofuel generation without additional requirements of land, water, or nutrients. However, there are challenges related to mass cultivation and downstream processing in realizing this vision.

Lipid generation at mass scale from the algae grown in wastewater requires understanding of algal species assemblages, enhancement of biomass accumulation of viable species, enumeration, quantification, and lipid profiling. Algal growth has been monitored through the measurement of cell densities with direct cell count, optical density (OD) and estimation of chlorophyll, volatile

solids, and so on. Factors such as nutrient uptake, cell development, cell division, increment in biomass, and lipid accumulation help evaluate cellular responses to local environment conditions. Cell composition and percentage fraction with substrate accumulation are best analyzed through GC–MS for lipid quantitation and composition analysis (Akotoa et al. 2005). Objectives of the study are to evaluate the scope of euglenoides—*L. ovum* predominantly grown in wastewater lagoons for mass biofuel production. This involves

i. Assessment of growth of euglenoides—*L. ovum* collected from lagoons fed with municipal wastewater;
ii. Investigation of lipid content and the composition of lipids; and
iii. Potential of algae-based biofuel in meeting the regional energy demand.

1.3.1 Materials and Methods

1.3.1.1 Study Area

Mysore lies between 12°9′ and 11°6′ latitude and 77°7′ and 76°4′ longitude in Karnataka, with the elevation of 650 m above mean sea level with a population of 1.2 million (in 2001). The temperature ranges from 14°C to 34°C and the monsoon showers starts from the end of May through October. The city is blessed with rivers like Cauvery for domestic water consumptions. Part of the city is dependent on groundwater for various purposes.

The wastewater generated in Mysore city due to gravity finds its way into three valleys—northern region leading Kesere valley, the southeastern to the Dalvai tank valley, and southwest to the Malalvadi lake valley. The northern drainage system leads to 30.0 MLD sewage treatment plant (STP; powered by aerators) at Kesare Village, Mysore. The southwestern drainage flow connects to the Vidyaranyapuram STP (67.65 MLD), which uses organic microbe-mix inoculum's (OS-1 and -2) for treatment before entering Dalvai kere. Further south is the STP at Rayankere (60 MLD), H. D. Kote Road. The study area (Figure 1.5) is essentially the catchment slope of the feeder to Dalvai lake. It lies between 12.273681° and 12.270031° latitude and 76.650737° and 76.655947° longitude at the foothills of Chamundi hills. The STP is also surrounded by numerous solid waste dumps. The effluent of the STP flows through the Dalvai kere and then traverses about 20 km before reaching Kabini river. The flow is considered laminar, the residence time of wastewater in the facultative ponds is 11.8 days and in maturation ponds is 2.5 days. The lagoon at Vidyaranyapuram consists of facultative (2, each of 5.15 ha spatial extent and depth) and maturation ponds (2 of 2.5 ha each).

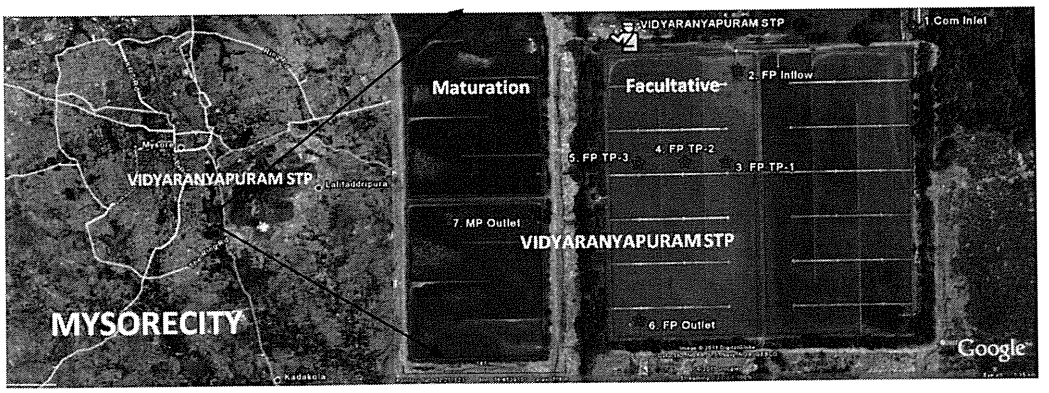

FIGURE 1.5 Sampling site—Vidyaranyapuram STP.

1.3.1.2 Sampling: Algal Screening, Selection, and Densities

For the biomass growth studies, the wastewater samples collected from the STP were filtered and used in the growth experiments. Algal samples were collected from anaerobic and aerobic regions of the facultative ponds as well as various locations of the inflows, outflows, and maturation ponds. The abundance of various species was calculated. The algal samples were identified using standard keys (Prescott 1964) based on their external appearance, color, morphological features, size, habitat, orientation of chloroplast, cellular structure and pigments, and so on. The wastewater samples collected were concentrated by centrifuging 15 ml volume. Algae were enumerated using three replicates of 20 μL of the concentrated sample through microscopic observations. Samples collected from the field were weighed for total dry weight. Quantification for unit area/volume was done taking 10-L volume for microalgae.

1.3.1.3 Characterization of the Growth Environment and Water Quality

Water samples (1 L) were collected from the sampling locations between 9 and 12 a.m. Sampling was always from the sunny side of the banks/platform so as to avoid shaded areas. pH, water temperature, electrical conductivity, total dissolved solids (TDSs), salinity, dissolved oxygen (DO), dissolved free carbon dioxide (free CO_2), and turbidity were measured on-site using the standard methods. In addition, visual clarity/transparency of the lake/STP wastewater was measured with Secchi disk. Water samples collected were analyzed for the various estimations of carbon, nitrogen and its various forms, and phosphorus according to standard methods (APHA 1998).

1.3.1.4 Harvesting of Algal Biomass

Algal biomass was collected from the wastewater ponds with buckets of 2.5-L capacity. The higher settling rate of *L. ovum* helped concentrate the algae, which were then transferred to the laboratory and carefully washed. After microscopic analysis, the samples were washed thrice thoroughly with deionized water and were concentrated by centrifuging at 5000 rpm for 20 min. The pellet was scrapped carefully using spatula and was exposed to drying at room temperature. Samples were preserved through refrigeration till further use.

1.3.1.5 Monitoring the Growth

Conducive growth environments were created for the cultivation with adequate sunlight and provisions for gaseous exchanges to stimulate the growth of the algae *L. ovum* collected and isolated from the wastewater ponds. The isolated algae *L. ovum* were grown, with the modified Chu's media with the composition: $Ca(NO_3)_2$ (20 g/L), K_2HPO_4 (2.5 g/L), $MgSO_4 \cdot 7H_2O$ (12.5 g/L), Na_2CO_3 (12.5 g/L), Na_2SiO_3 (10 g/L), $FeCl_3$ (0.4 g/L), and trace metal solution H_3BO_3 (2.48 g/L), $MnSO_4 \cdot H_2O$ (1.47 g/L), $ZnSO_4 \cdot 7H_2O$ (0.23 g/L), $CuSO_4 \cdot 5H_2O$ (0.1 g/L), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.07 g/L), $Co(NO_3)_2 \cdot 6H_2O$ (0.14 g/L), and finally the vitamin mix thiamine, HCl (Vit. B_1) (50 mg/L), biotin (Vit. H) (0.5 g/L), and cyanocobalamin (Vit. B_{12}) (0.5 g/L). A control was also prepared without any inoculations.

The wastewater collected from the influent of the lagoon was centrifuged, filtered, and sterilized for the growth studies. The initial pH was adjusted to 7.3 and the media was sterilized for 30 min before the growth experiments. The experiment was performed in conical flasks, each containing 100 mL of the wastewater media. The algal growth, that is, biomass growth, was measured every 24 h with a spectrophotometer (HACH, DR-2800) at 658 nm, using deionized water as the blank control. Probable relationship of biomass with the OD is given by

$$\text{Dry weight (g/L)} = 0.9712 \times \text{OD}_{658} \quad (r = 0.9973)$$

Approximately 10 mL of each sample was used and measured in 2.5-cm cuvettes. The conical flasks were agitated by hand prior to measurement in order to prevent settling of the algae. These experiments were performed in the batch mode for 9 days, as the retention time of the wastewater

facultative pond in the sewage ponds at Mysore was about 9 days. During this period, algal species were analyzed by OD measurements and also by direct cell counting method for obtaining the cell densities through conventional microscopy. These parameters were kept constant during the growth periods. Environmental scanning electron microscope (ESEM) Quanta was used for imaging of *L. ovum* cells after fixation with glutaraldehyde.

1.3.1.6 Spectral Signature and Biochemical Composition Analysis by ATR-FTIR

Fourier transform-infrared (FTIR) spectroscopy was used to study the macromolecular composition of algal biomass during the initial lag phase, the intermediate exponential phase, and final stationary phases of the *L. ovum* cell culture. Attenuated total reflectance (ATR-FTIR) spectra were collected on a Bruker Alpha Spec Instrument. The dried algal cells were pressed against the diamond cell prior to scanning. The finely powdered cellular extracts from *L. ovum* sp. were observed for their functionalities in the spectrogram. The spectra were collected in the Mid IR range (128 scans) from 4000 to 800 cm^{-1} (at a spectral resolution of 2 cm^{-1}) and data were analyzed using Origin Pro 8 SR0, v8.0724 (B724) with an initial base line correction and scaled up to amide I_{max}. The peaks with each of the spectral curves were carefully fitted and calculated. The changes in the carbohydrate, protein, lipids, and phosphates of the algal cells were determined from the variations in the intensities of specific bands of the FTIR spectrograms (Giordano et al. 2001; Murdock and Wetzel 2009).

1.3.2 Lipid Extraction and Analysis

1.3.2.1 Lipid Extraction

The algal cells were lyzed by sonication using an ultrasonic bath (frequency 35 kHz) for 45 min after concentration. The lipids were extracted from the solvent mixture as per modified Bligh and Dyer's (1959) method. One gram of dry algal biomass in triplicates was mixed with a solvent mixture of chloroform and methanol in a ratio of 1:2 (v/v). The organic chloroform layer was separated and was evaporated using a rotary evaporator fixed to a water bath maintained at 60°C. The various lipid classes were separated by thin-layer chromatography (TLC). The solvent system used for elution of lipids was a combination of petroleum ether:diethyl ether:acetic acid in a ratio of 70:30:1 (v/v) (Pal et al. 2011). Bands were visualized after staining the TLC plates with iodine vapors. The triacylglyceride (TAG) layer was immediately and carefully scrapped out and was processed for total lipid extraction. The fatty acids were analyzed by methylation of lipid samples using boron trifluoride–methanol (BF_3–MeOH), where BF_3–MeOH converts fatty acids into their methyl esters in a couple of minutes. The extracted samples were heated at 60°C for 15 min followed by cooling in ice bath for 5 min and then 1 ml of water and hexane were added, respectively. After settling, the top hexane layer was removed and washed using anhydrous sodium sulfate for further purification. The samples prepared were analyzed through the gas chromatography column. The methyl esters formed were assessed via gas chromatography (GC) and identified using mass spectroscopy (MS) on the basis of their retention time and abundance.

1.3.2.2 Fatty Acid Composition Using GC–MS

Fatty acid composition was analyzed through gas chromatography (Agilent Technologies 7890C, GC System) using detection by mass spectrometry (Agilent Technologies 5975C insert MSD with Triple-Axis Detector). The injection and detector temperatures were maintained at 250°C and 280°C, respectively (ASTM D 2800). One microliter of the sample was injected into the column, whose initial temperature was maintained at 40°C. After 1 min, the oven temperature was raised to 150°C at a ramp rate of 10°C min^{-1}. The oven temperature was then raised to 230°C at a ramp rate of 3°C min^{-1} and finally it was raised to 300°C at a ramp rate of 10°C min^{-1} and this temperature was maintained for 2 min. The methylated sample was loaded onto the silica column, with helium gas as carrier in splitless mode. The total run time was calculated to be 47.667 min. Fatty acids were identified by comparing the retention time obtained to that of known standards.

1.3.3 Scope for Biofuel as a Viable Energy Source in Cities of Karnataka

Estimates indicate that 40,700–53,200 L of oil/ha (max: 3,54,000 L/ha/year) of biofuel can be produced from algae from different regions having varying insolation capacities (Chisti 2007; Weyer et al. 2010). The density of the oil is about 918 g/L and is similar to the soybean oil (Weyer et al. 2010). Yield of conversion of algal crude lipid to biodiesel is about 70%–90% (Amin 2009) and a maximum of 98% (Chisti 2007). The heating value of the algal oil is about 42 GJ/ton (Amin 2009). Energy demand for regional transportation sector in Karnataka state has been compiled from the published government reports (State of Environment Report 2003).

1.3.3.1 Distribution, Morphological Features, and Cellular Characteristics of *L. ovum*

L. ovum indicative of higher organic loads (Affan et al. 2005) are unicellular flagellates belonging to the class Euglenozoa. During the study periods, *L. ovum* species were found at a lower N:P ratio ranging from 3.6 to 5.2, at a considerable organic load and at slightly acidic pH.

The body of the cell is more or less oval, and sometimes cylindrical and chloroplasts are numerous and discoidal in shape. Many storage bodies, that is, paramylum, were observed which were large (6–8 μm) and ring-shaped. The cells were 80–100 μm size in dimensions. These characteristics are provided in Table 1.10. These species normally found at lagoons, wastewater-treatment lagoons, ditches, swamps, tanks and reservoirs, small ponds, shallow sea bays, acidic lakes (Wollman et al. 2000), brackish waters (John et al. 2002), and also in well-aerated systems with moderate organic loads (Subakov-Simi et al. 2008). In the present study, these species were predominant in wastewater regions with higher organic regime lagoons.

1.3.4 Nutrient Requirements and Growth Conditions

1.3.4.1 Nutrient Concentrations and *L. ovum* Growth at Wastewater-Treatment Units

A slightly lower pH value during the sampling period were conducive for the predominance of *L. ovum*. However, with an increase in pH, the algal species richness generally increases, while diversity and abundance are low at lower pH (Wollmann et al. 2000).

TABLE 1.10
***L. ovum* Cellular Characteristics in Wastewaters**

Cell Characteristics	Values
Cell count (C)	8.74×10^5 cells/mL
Cell shape: average ratio of length to width of individual cells	Round: diameter 80–100 μm
Cell volume: the real volume of a million cells, in mm^3	33,493 μm^3
Packed cell volume (Vc): centrifuged volume in mL of the cells contained in 1 L of the sample after centrifugation at 5000 rpm	2.5 mL
Cell weight: dry weight of the cells in μg per million cells	0.48 mg/million cells
Cell density: dry weight of the cells in mg/m^3 of real volume	640×10^3 mg/m^3
Cell index: the packed volume per million cells (Vc/C)	2.86×10^{-3} m^3
Cell color	Pea green to bottle green
Cell types (stages)	Round, ovoid (mature)
Cell storage product	Starch: paramylon bodies (ring-shaped)
Bio-volume: volume of cells compared to volume of water they are occupied in 1 μm^3	0.03 μm^3
C (%): content in g/100 g of the cell dry weight	34.99
N (%): content in g/100 g of the cell dry weight	3.98
P (%): content in g/100 g of the cell dry weight	0.94

Their intensive growth of *L. ovum* is indicative of its ability to grow at lower pH with higher organic loads. The very high count is due to lower washout rate, with the longer retention period of the facultative ponds (10 days). In addition, under higher organic loads, some *Euglena* sp. (e.g., *Euglena gracilis*) through heterotrophy influences H^+ concentration, resulting in a marked decrease in water pH (Yamane et al. 2001). The lower DO levels have related to more of oxygen-demanding materials such as algal biomass, dissolved organic matter (DOM), ammonia, and sediment (Sanchez et al. 2007; Mahapatra et al. 2011a,b,c). Euglenoides can thrive in hypoxic, anoxic, and anaerobic environments as they are facultative anaerobic genera (Castro-Guerrero et al. 2005) and decrease in the hypoxic conditions (Moreno-Sanchez et al. 2000). Higher anoxygenic photosynthesis and high algal biomass for mixotropic culture of *euglenoides* reported here are similar to the earlier reports (Rosenberg 1963; Yamane et al. 2001; Ruiz et al. 2004). It is observed that the euglenoides were present in both anoxic and hypoxic conditions, and this could be a result of the switchover of the mode of metabolism. Similar cases have also been found in earlier studies (Tittel et al. 2003).

The physicochemical parameter of the growth environment of *L. ovum* in wastewaters is provided in Table 1.11. Among the various habitat parameters analyzed, the pH and DO were lower. The concentration of soluble orthophosphates reached 8.73 mg/L. Phosphorus, which regulates/limits the algal growth, is one of the most important nutrients. Higher growth of *L. ovum* species should involve higher uptake rates of phosphorus that should decrease the P concentration. But because of anoxic conditions, the sediment-bound P might have been released (Donnelly et al. 1998) that would be available to the euglenoides observed in the present study. Higher concentrations of N species as ammonium–N play a major role in the proliferation of euglenoid species (Duttagupta et al.

TABLE 1.11
Physicochemical Parameters of the Growth Environment of *L. ovum*

Parameters	Mean	±St. Dev.
pH	7.03	0.20
Temperature (°C)	24.60	2.68
Electrical conductivity (μS/cm)	1117.40	277.70
TDSs (mg/L)	649.33	156.02
Total suspended solids (mg/L)	588.00	56.00
Turbidity (NTU)	213.21	43.44
DO (mg/L)	0.22	0.35
Free CO_2 (mg/L)	38.59	12.88
COD (mg/L)	374.50	24.48
BOD (mg/L)	262.58	33.29
Nitrates (mg/L)	0.43	0.08
Ammonia (mg/L)	58.00	14.00
TKN (mg/L)	77.00	12.00
Phosphates (mg/L)	8.73	1.26
Total phosphates (mg/L)	12.20	4.60
Alkalinity (mg/L)	370.00	77.90
Total hardness (mg/L)	287.00	97.66
Calcium (mg/L)	68.00	15.00
Magnesium (mg/L)	26.00	6.60
Chlorides (mg/L)	176.16	116.35
SAR	18.75	3.79
TOC (mg/L)	102	5.9
Sodium (mg/L)	274.02	42.04
Potassium (mg/L)	26.75	13.02
ORP (mV)	−141.33	66.48

2004). A number of studies conducted earlier reveals that water bodies with high levels of organic compounds, for example, fish or sugar factory ponds (Lungu and Obuh 2004), municipal and dairy wastewaters (Bernal et al. 2008), and industrial wastewaters (Veeresh et al. 2010), had high population densities of euglenoids. The lower N:P (3.6–5.2) suggests P accumulation in the systems and is indicative of N-limiting conditions.

Euglenoides are well known for their sustained heterotrophic growth in the dark conditions (Ogbonna and Tanakaah 1998). It is observed that the regions with higher nutrient concentrations had higher euglenoid cell densities. Electrolyte concentrations during the higher growth periods were moderate (Table 1.11). *L. ovum* occurrence at a very high salinity (i.e., >3000 mg/L) and higher alkaline conditions (Wen and Zhi-Hui 1999) has been reported. This suggests the variability of the habitat and environmental conditions favorable for *L. ovum* in lagoons with varying conditions, which help in the nutrient recovery while accumulating lipid. Peptones are necessary as nitrogen sources in the darkness (Lwoff 1932) and studies have revealed that the euglenoides like *E. anabaena* and *E. deses* grow with inorganic N and *E. gracilis* can selectively take both inorganic and organic sources as N source (Hall and Schoenborn 1938; Schoenborn 1942). In this study, the euglenoides were found in the anoxic regions and are anticipated to feed on peptone type of protein, that is, N sources from the wastewaters.

1.3.4.2 Monitoring of Algal Growth

Algal growth studies were monitored through OD measurements at 658 nm. Growth of biomass (OD) was measured daily with the spectrophotometer. OD values showed that there was a considerable increase in the algal density with time (Figure 1.6). The maximum algal growth was found to be 470 mg/L at the end of the 7th day. *L. ovum* species grown in the wastewater media was under the lag period for the initial 3 days (due to the change in the growth environment), which picked up from the 4th day onwards, evident from a rapid growth of cell mass and an increased cell density due to better acclimatization and nutrient uptake. In contrary, algae grown in Chu media have higher lag period and the biomass increased during the 5th and 6th days. Similar studies using Chu media report maximum growth of 400 mg/L for *Botryococcus braunii* after the 11th day in batch systems (Sawayama et al. 1994). The growths of the algae were highest during the 7th day (3.67×10^5 cells/mL—OD 0.475), which started decreasing during the 8th day. Studies with *Chlamydomonas reinhardtii* grown in the influents, center, and effluents of municipal wastewaters showed higher biomass productivities of 2 g/day (OD 2.5) with the peak cell concentrations of 5.4×10^7 cells/mL in flask cultures with the cells reaching their exponential stage at the 7th day (Kong et al. 2010). Algal cultures grown in Chu media show the highest densities during the 6th day after which the densities started to decrease steeply, which could be attributed to lack of organic carbon in the medium. Studies have also reported reduced cell densities of *Haematococcus pluvialis* cultured in Chu media compared to the other media's (Fabregas et al. 2000).

Comparison of cell densities of algae grown in Chu and wastewater with the control is given in Figure 1.7. During the initial periods after the cell inoculations, the algal cells increased faster in

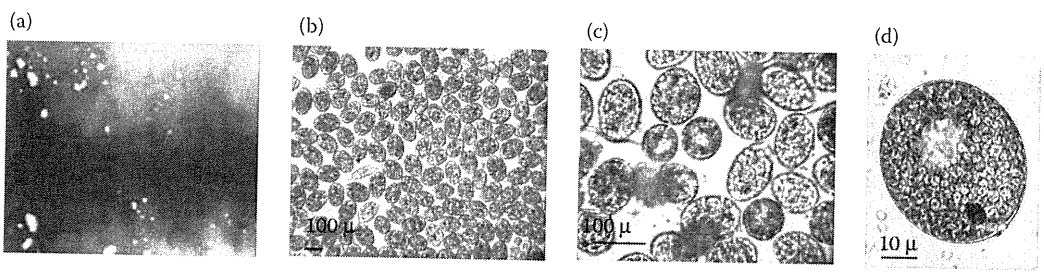

FIGURE 1.6 (a) *L. ovum* proliferation in the facultative ponds, (b) shape, (c) size, and (d) densities of *L. ovum* (at various levels of magnification).

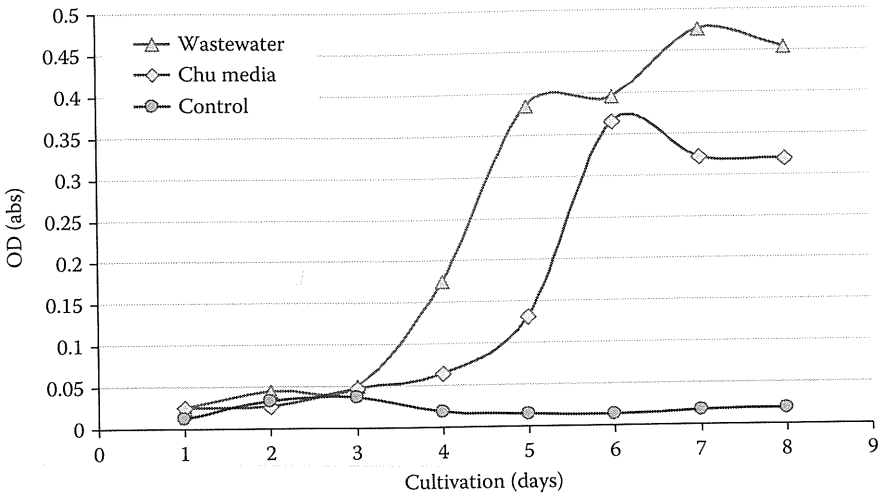

FIGURE 1.7 Comparative assessment of cell densities in diverse media.

the Chu media due to changes in the environment and nutrient conditions. During the lag phases, the cells were in clumps (i.e., attached to each other) due to mucilaginous secretions under stress conditions. Cell densities were higher on 6th and 7th days, with a higher biomass index due to high density of matured population.

The FTIR spectra of *L. ovum* biomass at initial (lag phase), intermediate (exponential phase), and final (stationary phase) stages of the culture are illustrated in Figure 1.8. The region 3300–3000 cm^{-1} is characteristic for C–H stretching vibrations of C≡C, C=C, and some aromatic hydrocarbons; on the other hand, the regions between 3000 and 2700 are assigned for C–H stretching vibrations of $>CH_2$, $-CH_3$, C–H, and CHO functional groups (Giordano et al. 2001; Sigee et al. 2002;

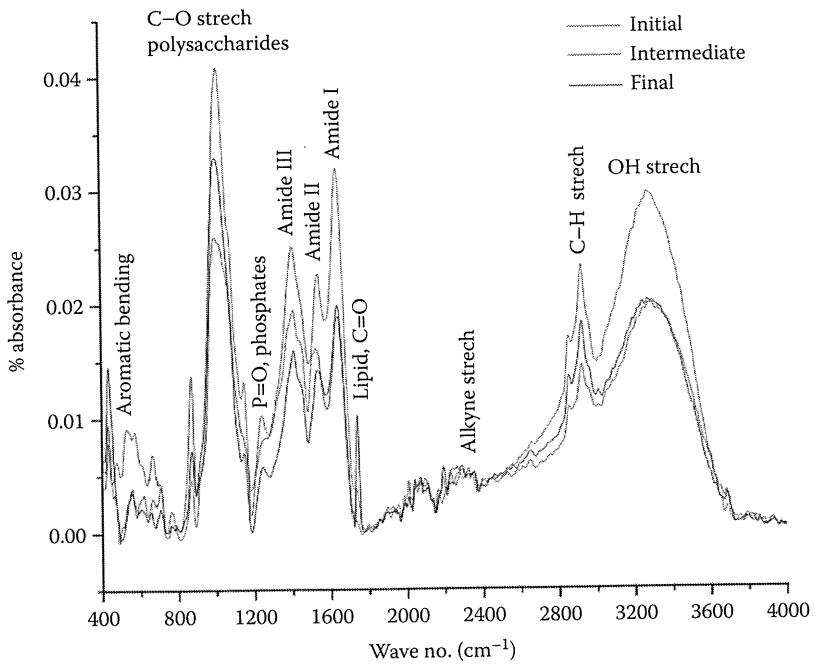

FIGURE 1.8 FTIR spectra of various stages of the cell growth.

Dumas and Miller 2003). The bands present between 3600 and 3300 cm^{-1} are due to the presence of unsaturates (olephinic C–H stretching vibrations). The band present at ~1640 cm^{-1} indicates the presence of C=O of lipids/esters (Sigee et al. 2002; Stehfest et al. 2005). The bands between 1700 and 1500 cm^{-1} indicate proteins. As depicted in Figure 1.8, peaks at ~1640 cm^{-1} are characteristics of amide I bands (Dumas and Miller 2003), and are due to C=O stretching vibrations of peptide bonds (Backmann et al. 1996). These bands provide essential information regarding the secondary structure of proteins (Fischer et al. 2006). The peaks at ~1540 cm^{-1} are for amide II bands, attributed to N–H bending vibrations in proteins (Fischer et al. 2006). The clusters of peaks at ~1357–1423 cm^{-1} are attributed to the amide III bands. The bands present at ~1245 cm^{-1} are for the phosphates and polyphosphates present in the algal cells (Stehfest et al. 2005). The wave numbers from 1200 to 900 cm^{-1} indicate stretching vibrations of polysaccharides due to C–C, C–O, C–O–C stretch (Yee et al. 2004) and rocking vibrations of CH_3 and CH_2. The presence of bands at ~2925 and 2855 cm^{-1} is indicative of asymmetric and symmetric CH_2 starching in lipid.

During the culture experiment, the initial lag phase showed lower absorbances in the polysaccharide region (1001 cm^{-1}) and amides I (1640 cm^{-1}) and II (1527 cm^{-1}) regions due to constraints in acclimatization, and the absorbance intensities are illustrated in Table 1.12 and depicted in Figure 1.8. During this stage, the cells just begin to grow and multiply. A very small peak was observed in the band corresponding to lipids (1732 cm^{-1}) in the culture systems. The observations made during the exponential stages of the growth showed higher absorbances for polysaccharides, proteins, especially amide I and II, phosphates, and a slight increase in the lipid content as provided in Figure 1.8 and Table 1.12. However, during the stationary phase, that is, final stages, a distinct lipid band (~1740 cm^{-1}) was observed indicating lipid accumulation. The comparison of band intensities shows a conversion of carbon from carbohydrates and proteins into lipids. This is evident from the decreased intensities of polysaccharides and proteins. The major reasons for lipids accumulation at the later stages of growth are N and P limitations. This is in agreement with other studies (Sigee et al. 2002; Stehfest et al. 2005; Dean et al. 2007). There was not much of a change in amide I bands when the lag phases were compared with the final stages of algae during the growth. The protein content significantly decreased from the exponential stages to the final starvation stages indicating N stress that could be a possible reason for lipid accumulation. Marked changes in the intensities were observed at the various stages of cell culture indicating variable nutrient uptake, assimilation, and accumulation. The SEM image of *L. ovum* is depicted in Figure 1.9.

1.3.4.3 Lipid Composition by GC–MS Analysis

Total lipid content of *L. ovum* is 18.4 ± 1.04%, which is relatively lower compared to the earlier reports with euglenoides (Constantopolous and Bloch 1967). Algal species grown in batch mode in the wastewater has a total lipid content (of oil content):

a. 31% in *Scenedesmus obliquus* grown in secondary-treated municipal wastewaters (Martinez et al. 2000)
b. 25.25% in *C. reinhardtii* when cultured in all stages of municipal wastewaters (Kong et al. 2010)
c. 18% in *Botryococcus braunii* in municipal wastewaters (Orpez et al. 2009)
d. 9% in mixed population *Scenedesmus* sp. followed by *Micractinium* sp., *Chlorella* sp., and *Actinastrum* sp. in primary-treated wastewaters (Woertz et al. 2009)

Compared to these, the lipid content of *Chlorella* sp. grown in a semicontinuous mode in wastewaters was 42% with an effective COD, NH_3–N, and P removal (Feng et al. 2011). Fatty acid methyl esters (FAME) were separated according to the retention times in the column. The proportion of each fatty acid was calculated based on the peak area (area under the curve) and are provided in Table 1.13, which illustrates the composition of the fatty acid (saturates and unsaturates). The total saturated fatty acids (68.1%), monounsaturates (19.34%), polyunsaturates (12.96%), and fatty

TABLE 1.12
Band Assignments for Infrared Spectra

	Initial Lag Phase		Intermediate Exponential Phase		Final Stationary Phase				
No.	Intensities	Wave Number (cm^{-1})	Intensities	Wave Number (cm^{-1})	Intensities	Wave Number (cm^{-1})	Wave Number Range (cm^{-1})	Main Groups	Band Assignments
1	0.01997	3295	0.02972	3275	0.02027	3274	3029–3639	OH Amide A	Water ν(O–H) stretching Protein ν(N–H) stretching (amide A)
2	0.01418	2922	0.02329	2923	0.01831	2925	2809–3012	C–H	Lipid–carbohydrate Mainly $\nu_{as}(CH_2)$ and $\nu_s(CH_2)$ stretching
3	0.00101	1732	0.00368	1732	0.01032	1740	1763–1712	Lipids	Fatty acids ν(C=O) stretching of esters
4	0.01896	1640	0.03198	1639	0.01988	1640	1583–1709	Amide I	Protein amide I band: Mainly ν(C=O) stretching
5	0.01613	1527	0.02267	1540	0.01421	1535	1481–1585	Amide II	Protein amide II band: Mainly δ(N–H) bending and ν(C–N) stretching
6	0.02511	1408	0.01952	1409	0.01596	1411	1357–1423	Amide III	Protein $\delta_{as}(CH_2)$ and $\delta_{as}(CH_3)$ bending of methyl, lipid $\delta_s(N(CH_3)_3)$ bending of methyl
7	0.00824	1256	0.01027	1242	0.00579	1245	1190–1350	Phosphates	Nucleic acid (other phosphate-containing compounds as phospholipids) $\nu_{as}(>P=O)$ stretching of phosphodiesters
8	0.02596	1001	0.04088	1017	0.03295	1006	980–1072	Saccharides	Carbohydrate ν(C–O–C) of polysaccharides

Source: Adapted from Giordano M et al. 2001. *J Phycol* 37:271–279; Sigee DC et al. 2002. *Eur J Phycol* 37:19–26; Benning LG et al. 2004. *Geochim Cosmochim Acta* 68:729–741; Dean AP et al. 2010. *Bioresour Technol* 101:4499–4507; Dean AP, Martin MC, Sigee DC. 2007. *Phycologia* 46:151–159; Stehfest K, Toepel J, Wilhelm C. 2005. *Plant Physiol Biochem* 43:717–726.

Note: ν_{as}, asymmetric stretch; ν_s, symmetric stretch; δ_{as}, asymmetric deformation; δ_s, symmetric deformation.

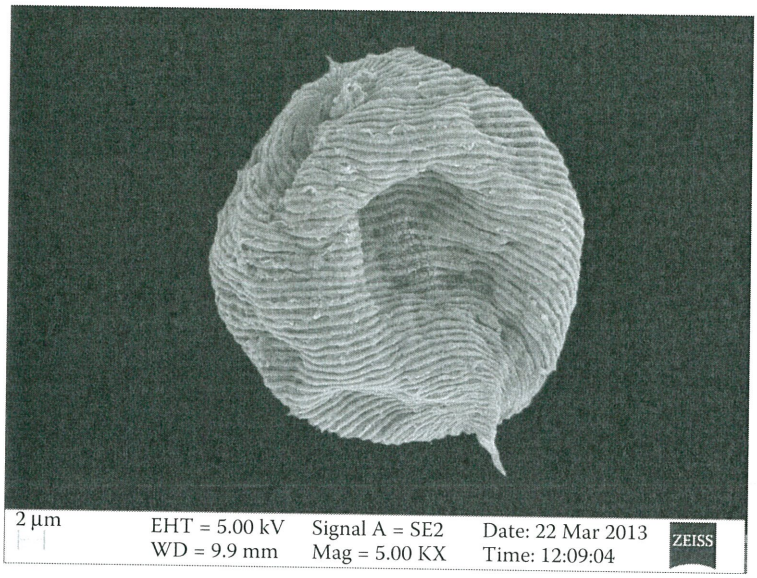

FIGURE 1.9 SEM image of *L. ovum*.

acids (72.94%) reflect properties of biodiesel. Important fatty acids from biofuel perspective of *L. ovum* using the ultrasonication techniques are given in Figure 1.10. The present analysis showed 15 different types of fatty acids. The fatty acid composition has higher percentage of palmitic acid [C16:0] 30.32% > oleic acid [C18:1(9)] 12.94% > stearic acid [C18:0] 12.04% > methyl tetradecanoate [C14:0] 10.38% > pentadecanoic acid [C15:0] 8.97% > linoleic acid [C18:2(9,12)] 8.44% > linolenic acid [C18:3(9,12,15)] 4.52%. These results are in agreement with earlier studies (Knothe 2008), with palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) are the most common fatty acids for providing a reasonable balance of fuel properties. The chromatogram derived from the gas chromatography and mass spectrogram (GC and MS) analyses of the fatty acids for *L. ovum* is elucidated in Figure 1.11, which highlights the peaks of the fatty-acid methyl esters.

These results indicate that *L. ovum* constitute a better candidate for biodiesel production, due to higher content of requisite fatty acids (68.1% saturates: Table 1.13) comparable to biodiesel properties. Similar findings were also reported earlier (Rasoul-Amini et al. 2009), and also algal oils having high polyunsaturated fatty acids with four or more double bonds (Damiani et al. 2010). Higher content of saturated fatty acids provides an excellent cetane number (better ignition quality) and oxidative stability to biodiesel (Chinnasamy et al. 2010). The present study reveals that appropriate optimization of euglenoid microhabitat, development, growth conditions, and lipid composition to enhance the net productivity from wastewater systems would ensure a sustained energy supply while treating wastewater at decentralized levels which foster human welfare.

1.3.4.4 C Sequestration in Wastewater Algae

The average biomass productivity found in the Mysore pond systems ranged from 10 to 20 g/m^2/day and considering the surface BOD loading 400–900 kg/ha/day, the CO_2 sequestration potential in the native *L. ovum* species was calculated to be 1.23–1.48 tons CO_2-eq./ha/day providing an annual C sequestration of ~490 tons CO_2-eq./ha and from this ~10 tons CO_2-eq./ha/year is the C sequestered and transformed to lipids that can be used effectively as a biofuel. This unique way of C sequestration and transformation into valorizable algal biomass with ~20% lipids paves path for new avenues for energy sustainability with adequate reduction of wastewater C footprint. The algal communities also create aerobic environment, thus abating the formation of methane which is a potential GHG.

TABLE 1.13
Fatty Acid Profile of *L. ovum* through GC–MS Analyses

CN:U	FAME	Peak Area	Corr. Area	Corr. %max	Retention Time	%Comp.
C 10:0	Decanoic acid, methyl ester	22,363	60,004	0.31	14.137	0.09
C 11:0	Undecanoic acid, methyl ester	35,981	87,061	0.45	16.561	0.14
C 12:0	Dodecanoic acid, methyl ester	186,635	527,647	2.73	19.01	0.83
C 13:0	Tridecanoic acid, methyl ester	611,028	2,006,749	10.37	22.054	3.14
C 14:0	Methyl tetradecanoate	1,795,343	6,625,155	34.24	25.291	10.38
C 15:0	Pentadecanoic acid, methyl ester	1,438,245	5,578,805	28.83	28.537	8.97
C 16:0	Hexadecanoic acid, methyl ester	622,964	2,439,440	12.61	31.038	30.32
C 16:1(7)	7-Hexadecenoic acid, methyl ester	54,675	213,817	1.11	31.791	0.34
C 16:1(9)	9-Hexadecenoic acid, methyl ester	4,012,960	9,349,604	0.00	31.791	3.82
C 17:0	Heptadecanoic acid, methyl ester	217,340	1,333,190	6.89	34.836	2.09
C 17:1(10)	*cis*-10-Heptadecenoic acid, methyl ester	372,136	1,428,305	7.38	34.069	2.24
C 18:0	Octadecanoic acid, methyl ester	1,028,784	4,427,325	22.88	36.861	12.05
C 18:1(9)	9-Octadecenoic acid, methyl ester	1,875,793	7,746,598	40.03	37.217	12.94
C 18:2(9,12)	9,12-Octadecadienoic acid, methyl ester	249,646	958,305	4.95	37.478	8.44
C 18:3(9,12,15)	9,12,15-Octadecatrienoic acid, methyl ester	705,164	2,881,726	14.89	37.071	4.52
					Saturated fatty acids (saturates)	68.01
					Monoenoic fatty acids (mono-unsaturated fatty acids)	19.34
					Polyenoic fatty acids (poly-unsaturated fatty acids)	12.96
					C16–C18 (fatty acids important from biodiesel perspective)	72.94
					Total lipid content	18.48

1.3.5 Viability of Algae-Based Biofuel as an Energy Source in Karnataka

The current investigations reveal that resident algal populations growing in wastewater systems are not only aiding in treating wastewaters, but also ensure reliable substrate for biofuel generation in Mysore being one of the rapidly growing cities in Karnataka generates about 250 MLD of sewage (Table 1.14). Three treatment plants with the installed capacity of 150 MLD are in operation. The conventional wastewater treatment plants face the problem due to the lack of assured supply of

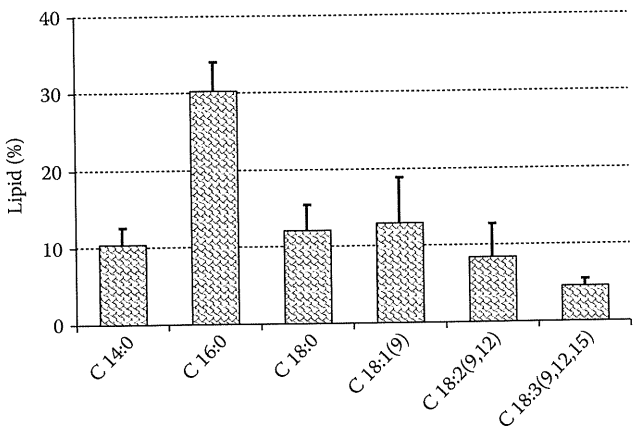

FIGURE 1.10 Comparative account of the major fatty acid composition in *L. ovum*.

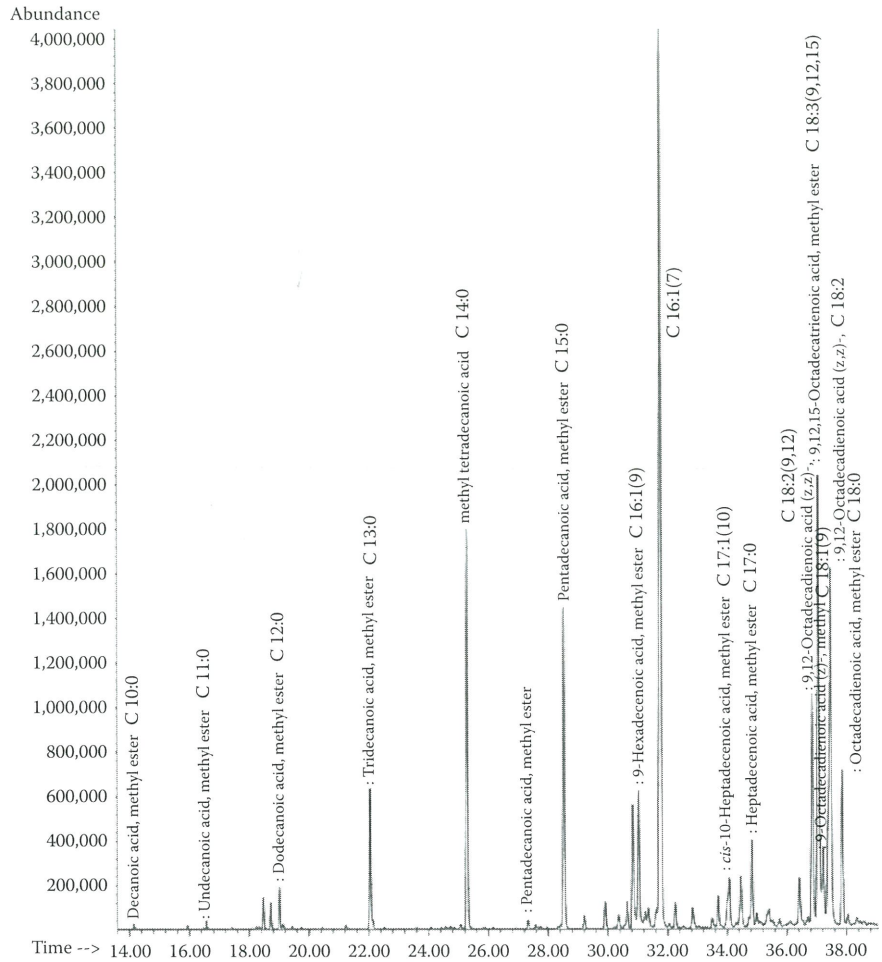

FIGURE 1.11 Chromatogram depicting peaks of major fatty acids in *L. ovum*.

electricity and also under capacity to handle the increasing load. However, algal population present at various unit processes of the treatment plants, that is, inflows, facultative ponds, maturation ponds, and the outflows, are aiding in the nutrient uptake or in the treatment. Very high densities of *L. ovum* were observed in the facultative ponds at a very high surface BOD loading 400–900 kg/ha/day with a retention time of 10–12 days. The area required to treat 100 MLD of wastewater is about 20 ha.

On an average, 640 mg of algal dry weight were derived from 1 L of the wastewater and the cells take roughly 7–8 days to reach the exponential growth stage with 4.4×10^5 cells/mL. At such cellular densities, the biomass productivities were about 250–300 tons/ha/year and about 18% of the dried algal biomass constitutes lipid which is about 40–50 tons/ha/year of lipid from *L. ovum*. Various studies conducted earlier have estimated the regional or local estimates of the potential of algal biofuel. Studies reveal that after allowing for maximum photorespiration the maximum yields of carbohydrate-type molecules are about 410 tons/ha/year at tropics (Williams and Laurens 2010), 182 tons/ha/year through culturing diatoms *Phaeodactylum tricornutum* in bioreactors (Fernandez et al. 1998), 60 tons/ha/year in raceway ponds at subtropics with *Pleurochrysis* sp. (Moheimani and Borowitzka 2007), 175 tons/ha/year bioreactor raceway pond systems (Chisti 2007), 50–300 tons/ha/year in closed photobioreactors (Sheehan et al. 1988), 22.87–137.25 tons/ha/year at about

TABLE 1.14
District-Wise Analysis of the Algal Biofuel Potential from Wastewaters

Districts	Total Geographic Area (Ha)	Population (Census Data 2011)	Per Capita Water Use (L) in the Region per Day	Wastewater Generation (MLD)	Total Wasteland (Ha)	Area Req. for Algal Pond (Ha)	Waste Land % to Total Geog. Area	Land Req. for Algal Ponds % to Waste Land	Algal Lipid Potential (Tons/Year)
Bagalkote	657,500	1,890,826	226,899,120	158.83	69,932	79.41	10.64	0.11	3964.38
Bengaluru Urban	219,000	9,588,910	1,150,669,200	805.47	6525	402.73	2.98	6.17	20,104.49
Belagavi	1,341,500	4,778,439	573,412,680	401.39	143,000	200.69	10.66	0.14	10,018.67
Bellary	841,900	2,532,383	303,885,960	212.72	109,120	106.36	12.96	0.10	5309.50
Bidar	544,800	1,700,018	204,002,160	142.80	36,127	71.40	6.63	0.20	3564.33
Bijapur	1,049,400	2,175,102	261,012,240	182.71	32,996	91.35	3.14	0.28	4560.41
Chamarajnagar	568,500	1,020,962	122,515,440	85.76	17,150	42.88	3.02	0.25	2140.59
Chikamagalur	720,100	1,137,753	136,530,360	95.57	60,676	47.79	8.43	0.08	2385.46
Chitradurga	844,000	1,660,378	199,245,360	139.47	119,096	69.74	14.11	0.06	3481.21
Davanagere	596,600	1,946,905	233,628,600	163.54	50,342	81.77	8.44	0.16	4081.96
Dharwad	423,000	1,846,993	221,639,160	155.15	14,508	77.57	3.43	0.53	3872.48
Gadag	465,700	1,065,235	127,828,200	89.48	37,237	44.74	8.00	0.12	2233.41
Kalaburagi	1,622,400	2,564,892	307,787,040	215.45	78,401	107.73	4.83	0.14	5377.66
Hassan	681,400	1,776,221	213,146,520	149.20	31,595	74.60	4.64	0.24	3724.10
Haveri	485,100	1,598,506	191,820,720	134.27	29,147	67.14	6.01	0.23	3351.49
Kodagu	410,200	554,762	66,571,440	46.60	8368	23.30	2.04	0.28	1163.14
Kolar	822,300	1,540,231	184,827,720	129.38	59,536	64.69	7.24	0.11	3229.31
Koppala	718,900	1,391,292	166,955,040	116.87	43,740	58.43	6.08	0.13	2917.04
Mandya	496,100	1,808,680	217,041,600	151.93	39,077	75.96	7.88	0.19	3792.15
Mangalore	484,300	2,083,625	250,035,000	175.02	20,889	87.51	4.31	0.42	4368.61
Mysore	626,900	2,994,744	359,369,280	251.56	20,430	125.78	3.26	0.62	6278.90
Raichur	682,800	1,924,773	230,972,760	161.68	74,544	80.84	10.92	0.11	4035.56
Shivamogga	846,500	1,755,512	210,661,440	147.46	39,178	73.73	4.63	0.19	3680.68
Tumakuru	1,059,800	2,681,449	321,773,880	225.24	99,718	112.62	9.41	0.11	5622.03
Udupi	359,800	1,177,908	141,348,960	98.94	25,710	49.47	7.15	0.19	2469.65
Uttara Kannada	1,029,100	1,436,847	172,421,640	120.70	57,639	60.35	5.60	0.10	3012.55
Bengaluru Rural	250,000	987,257	118,470,840	82.93	19,000	41.46	7.60	0.22	2069.92
Ramanagaram	331,500	1,082,739	129,928,680	90.95	20,000	45.48	6.03	0.23	2270.11
Total	1,917,9100	5,870,3342	7,044,401,040	4931.08	1,363,681	2465.54			123,079.77

30%–70% oil on a dry weight basis (Chisti 2007), 12.6 tons/ha/year for tropical countries, that are comparatively much lower than the optimal estimates (Rodolfi et al. 2008). In a recent study, the optimal yields of algal lipids at the tropics were estimated to be as low as 36–45 tons/ha/year (Weyer et al. 2010). The resident algal populations (*L. ovum*) isolated from the facultative lagoons with high organic loads help in wastewater treatment and also in biofuel generation. Replication of these systems in all urbanizing towns would aid as low-cost option for wastewater treatment while meeting the energy demand at local levels.

For effective algal growth and year-round productivity, the ponds have to be shallow (1 m) to reduce the shading effects caused by the higher cell densities. This requires 2.5 times more area than what is currently being practiced as area required for the facultative lagoons. These types of shallow systems will treat wastewaters and at the same time generate ample algal biomass for deriving energy. At such high productivities of 250 tons/ha/year, and assuming that entire wastewater treatment and consequent lipid generation would meet the transport and irrigation (diesel pump) energy requirement in rural area, the algal biofuel proves to be a sustainable option. Taking the per capita generation of wastewater as 80% of the total water consumption the daily generation of wastewater is 96 L/day/capita. The wastewater has to travel a certain distance to reach such community/regional algal ponds. As most of the advanced cities of India are connected to underground drainage (UGD), the chances of wastewater infiltration are low but pilferages cannot be ignored at the source points—on the way to the treatment units and while joining receiving waters. In the case of small cities, most of the wastewater generated is adequately allowed to infiltrate in pits and soak ways (Parkinson et al. 2008). The degree of infiltration varies based on the nature of soil types. The infiltration is higher with sand and silt type of soils (150 L/m^2/day).

During this travel, approximately 10% of the wastewaters are lost mainly by infiltration and to a smaller extent through evaporation. Accounting for all these losses, the wastewater generated in Karnataka state is around 4931 MLD with the land requirement of 2465 ha for implementing algae-based wastewater lagoons and subsequent lipid recovery. The total wasteland area in Karnataka is about 1.37 million hectares.

District-wise total area required for integrated algal pond-type treatment cum lipid recovery systems are given in Table 1.14 for Karnataka, a federal state in India. The percentage of land required for algal lipid production (only from wastewaters) is less than 1% of the total wastelands area required for catering the entire wastewaters of Karnataka with simultaneous biofuel generation except for Bengaluru, where the area required for high-detention algal ponds is about 6% of the wasteland. The district-wise algal lipid potential derived from wastewaters is elucidated in Figure 1.12. This illustrates that Bengaluru, Mysore, Belagavi, Kalaburagi, and Tumakuru have higher lipid potential (5000–20,000 tons/year; Figure 1.12). The petrol and diesel consumption was 5,09,918 and 22,98,370 tons (in 2003–2004), respectively (State of the Environment Report 2003). Estimates show that fuel oil consumption for the transport sectors in the state at present is estimated to be around 30,00,000 tons/year. To meet the energy requirement for the transport sector in the state, only 5% of the total wasteland (60,000 ha) area is required to build high-detention algal ponds, which paves a way for sustainable biofuel generation and wastewater treatment.

The present studies on resident algal population in the wastewater treatment ponds revealed the dominance of an euglenoid *L. ovum* through mixotrophy (both autotrophy and heterotrophy) at anoxic waters with higher organic loads and ammonium-N concentrations, where other algae fail to grow. The species isolated and grown in Chu media showed lower growth rates compared to the wastewaters that attained maximum biomass productivity at the 7th day. The total lipid content was found to be 18.6%. The fatty acid composition showed 68% saturates, 18% monounsaturates, and 13% polyunsaturates. The essential fatty acids from the biodiesel perspective were 76% showing the algae as potential qualifier for a biodiesel feedstock. The fatty acid profile of *L. ovum* demonstrated that palmitic acid (16:0), stearic acid (18:0), linoleic acid (18:1), methyl tetradecanoic acid (14:1), oleic acid (18:1), and linolenic acid (18:3) are major fatty acids of *L. ovum*, implying the potentials of microalgal lipids for biodiesel.

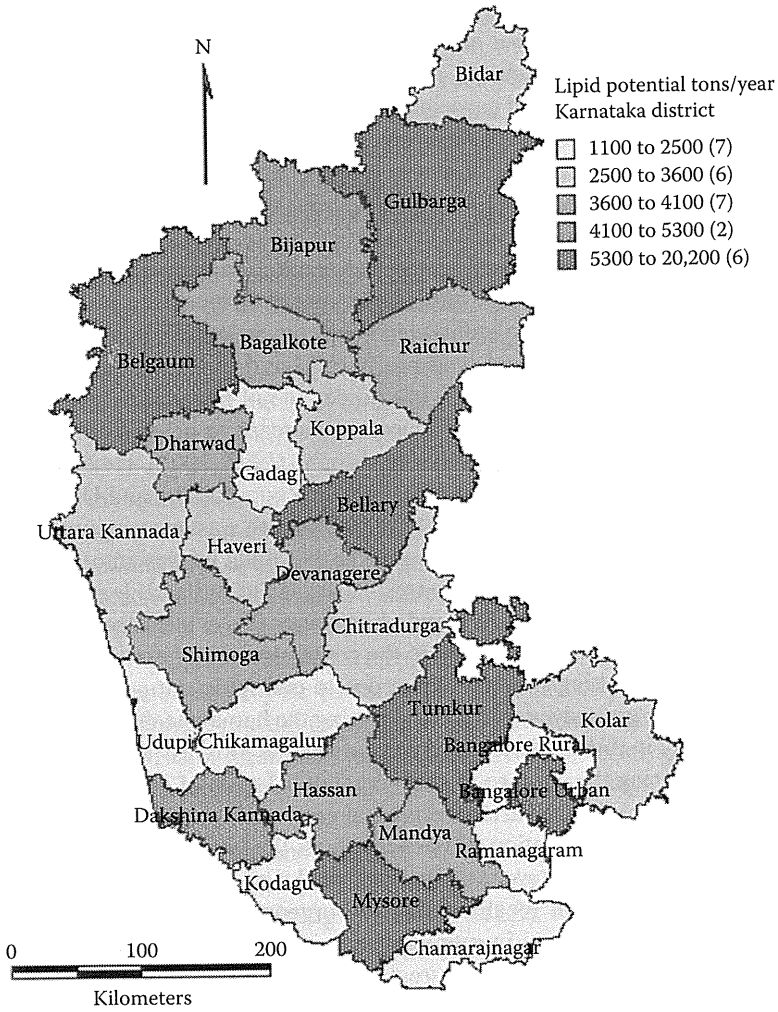

FIGURE 1.12 District-wise algal lipid potential in tons/year for Karnataka state.

The evaluation of the resident wastewater algae for the regional energy demand for transport showed positive results. With the current wastewater generation rate, about 26.78 tons/year of lipid can be generated from 1 MLD of wastewaters. The land required for the cultivation of wastewater-grown algae is calculated to be 0.5 ha/MLD. The lipid potential calculated at the maximum growth conditions was found to vary between 40 and 50 tons/ha/year due to seasonal variabilities. The biomass productivities can be further optimized in wastewater ponds to establish a sustainable biomass production process. This resident wastewater algae *L. ovum* can be grown in the wastewaters of the cities in Karnataka and simultaneously treat the wastewater and generate biofuel to meet the regional energy requirement.

The estimate of the potential of algae to replace vehicle fuels in the Karnataka state shows that an area corresponding to at least 5% of the total wastelands would be required to satisfy current fuel oil demand in the transport corridor. However, future fuel demand is most likely to be met from a combination of algal substrates at the end of each stage of processing by algal bio-refinery approach rather than only wastewaters as a solution.

The potentials of the existing resident wastewater algae, which otherwise cause a nuisance by their prolific outbreaks in eutrophied freshwater, can be harvested as resources. The large quantum

of terrestrial nutrients (sewage) getting into these waters are a sustainable nutrient feed for the algae for its growth and development. Further investigations have to be carried out to better understand the wastewater ecology, nutrient dependencies, mode of nutrition, and the growth microenvironment of the promising microalgae *L. ovum* and consequently optimizing the growth rates and lipid accumulation of these resident algae for integrated wastewater treatment and sustainable bioenergy generation.

1.4 INTEGRATED WETLANDS ECOSYSTEM TO MITIGATE CARBON EMISSIONS

Increased and unprecedented population growth has resulted in enormous stress on potable water from a daily consumption point of view and also in regard to increased wastewater generated by the city. Unplanned growth has led to radical land-use conversion of forests, surface water bodies, and so on with the irretrievable loss of land prospects. Land-use analyses show 584% growth in built-up area during the last four decades with the decline of vegetation by 66% and water bodies by 74%. Analyses of the temporal data reveal an increase in urban built-up area of 342.83% (during 1973–1992), 129.56% (during 1992–1999), 106.7% (1999–2002), 114.51% (2002–2006), and 126.19% (during 2006–2010) (Ramachandra et al. 2012; Ramachandra and Uttam Kumar, 2008).

Rapid urbanization in recent times has led to the mammoth wastewater generation. Untreated or partially treated wastewaters are fed to surface water that finds its way into groundwater sources. The sustained inflow of untreated or partially treated sewage to wetlands leads to the enrichment of nutrients such as carbon (C), nitrogen (N), and phosphorus (P), evident from the algae bloom and profuse growth of macrophytes. This has led to the contamination of existing water resources with pathogens and nutrients resulting in algal bloom due to eutrophic status of surface water, thereby contaminating the nearby groundwater sources affecting the human health. On the other hand, macrophytes grow profusely in this nutrient-rich environment and progressively cover the entire surface of the water body hindering the passage of sunlight and diffusion of gases to the underlying water layers. Absence of sunlight in these parts affects algal growth and photosynthetic O_2 generation and critically depletes the DO concentration and hence affects the local biota.

Treatment and disposal of wastewater generated in the neighborhood constitute key environmental challenges faced in urban localities due to burgeoning population in the recent decade. Nutrient-laden wastewater generated in municipalities is either untreated or partially treated and is directly fed into the nearby water bodies regularly, resulting in nutrient enrichment and algal blooms. Conventional wastewater-treatment options are energy- and capital-intensive apart from their inability to remove nutrient completely. In this backdrop, algal processes are beneficial and remove nutrients with carbon sequestration and resultant biomass production. Algae grow rapidly and uptake nutrients (C, N, and P) available in the wastewater (GOI 2008; Mahapatra et al. 2013a,b; Sharachchandra Lele et al. 2013) and hence are useful in nutrient remediation. Treatment of sewage and letting into wetlands would help in further treatment (removal of N, P, and heavy metals). This also prevents contamination of groundwater resources. Thus, wetlands provide a cost-effective option to handle sewage generated in the community and also help in addressing the water crisis in the region.

Microalgae and native macrophytes of the wetlands help in the treatment due to abilities to uptake nutrients and heavy metals. Techniques have been developed for exploiting the algae's fast growth and nutrient removal capacity (Karin 2006). The nutrient removal is basically an effect of assimilation of nutrients as the algae grow. Also, nutrient stripping happens due to high pH induced by the algae as in ammonia volatilization, phosphorus precipitation, and so on.

1.4.1 Wetlands/Algae Pond as Wastewater Treatment Systems

Wetlands aid in water purification (nutrient, heavy metal, and xenobiotics removal) and flood control through physical, chemical, and biological processes. When sewage is released into an environment containing macrophytes and algae, a series of actions takes place. Through contact with

biofilms, plant roots and rhizomes processes like nitrification, ammonification, and plant uptake will decrease the nutrient level (nitrate and phosphates) in wastewater (Garcia et al. 2010). Algae-based lagoons treat wastewater by natural oxidative processes. Various zones in lagoons function equivalent to cascaded anaerobic lagoon, facultative aerated lagoons followed by maturation ponds (Mahapatra et al. 2013b). Microbes aid in the removal of nutrients and are influenced by wind, sunlight, and other factors (Mahapatra et al. 2011b,c, 2013b).

The conventional wastewater treatment systems (sewage treatment systems) are expensive and require input of external energy sources (e.g., electricity, organic carbon) and chemical additives. These treatment systems generate concentrated waste streams necessitating environmentally sound disposal. There is an urgent need to develop innovative, environment-friendly, and cost-effective approaches for treating sewage generated in the community every day. Untreated sewage leads to the neighborhood contamination of land and water resources (groundwater). An easy way to check the sewage contamination is to test the level of nutrients (nitrates and phosphates). Nitrate is a substance that develops from organic waste. Algae convert nitrate into organic compounds (proteins) through photosynthesis in the presence of sunlight. Algae can exhibit growth rates that are higher than other plants due to their extraordinarily efficient light and nutrient utilization. By taking advantage of rapid availability of nutrient-enriched water, high solar intensity, and favorable microclimate for algal growth, higher densities of algae can be grown continuously that provides ample biomass and at the same time treats wastewater within a short period of time.

Algal bacterial symbiosis is very effective in these tropical conditions. Algae, the primary producers, generate O_2 (during photosynthesis) which aid in the efficient oxidation of organic matter with the help of the chemoorganotrophic bacteria. The type and diversity of the algae grown are potential indicators of treatment process (Mahapatra and Ramachandra 2013; Ramachandra et al. 2012; Mahapatra et al. 2013a,b; Mahapatra et al. 2014). And bacterial system disintegrates and degrades the organic matter, providing the algae with an enriched supply of CO_2, minerals, and nutrients.

The focus of the current investigation is to assess the efficacy of wetlands in Jakkur lake system. This has been done through water quality assessment (physico-chemical analysis) at various stages of the integrated wetland system consisting of STP (10 MLD), wetlands (with macrophytes), algal pond, and Jakkur lake (Figure 1.13). Nitrate and phosphate levels were monitored at various stages of wetland ecosystem.

Jakkur lake (Figure 1.13) situated at 13°04′N and 77°36′E, northeast of Bengaluru. Ten MLD STP is functional in this locality. Partially treated water is let into Jakkur lake through wetlands (consisting of emergent macrophytes and algae). Water samples were collected (Figure 1.13) from inlet (S6), outlets (S1, S2, and S3), middle (S4, S5, and S9), and at treatment plant outlets (S6 and S7) totaling nine locations. The treated water from the treatment plant passes through the wetlands to Jakkur lake.

1.4.2 Integrated Wetland System

Integrated wetland system at Jakkur consists of (i) treatment plant (treats sewage partially before letting to wetlands, (ii) constructed wetlands consisting of macrophytes, (iii) algal pond, and (iv) lake (Figure 1.13). Jakkur lake with wetlands is manmade and constructed about 200 years ago to meet the domestic and irrigation water requirement of Jakkur village located about 100 m southwest in the downstream of the lake (Figure 1.13).

1.4.2.1 Nutrients (Nitrates and Phosphates)

Nutrients essentially comprise of various forms of N and P that readily mineralize (inorganic mineral ions) to enable uptake by microbes and plants. Accumulation of nitrates and inorganic P induces changes in water quality and affects its integrity leading to higher net productivity. Nitrates in excess amounts together with phosphates accelerate aquatic plant growth in surface water causing rapid

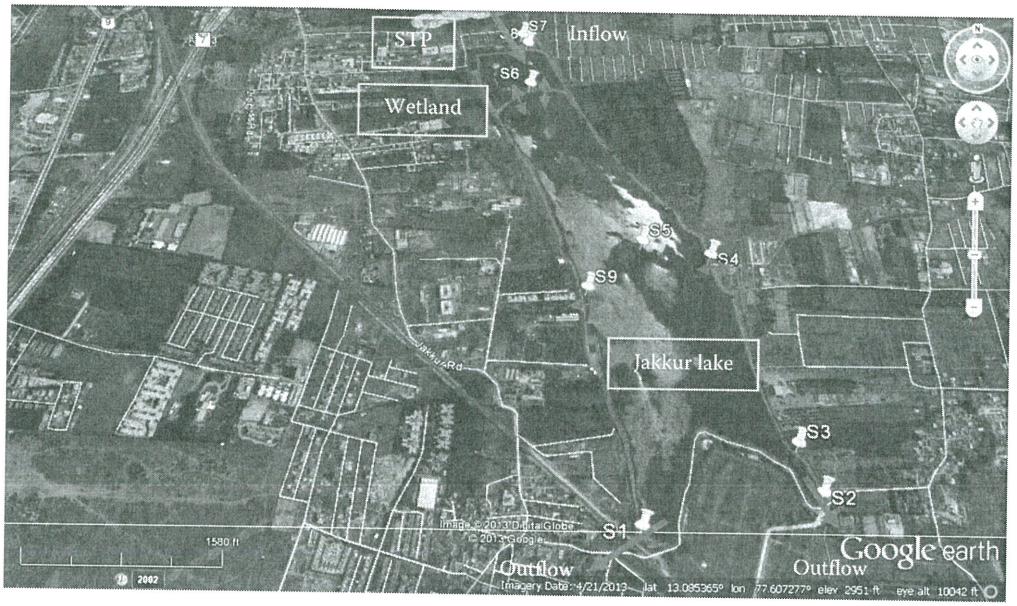

FIGURE 1.13 Water sampling locations in wetland system.

oxygen depletion or eutrophication in the water. Nitrates at high concentrations (10 mg/L or higher) in surface and groundwater used for human consumption are particularly toxic to young children affecting the oxygen-carrying capacity of red blood cells (RBCs) causing cyanosis (methemoglobinemia). In the current study nitrate values ranged from 0.2 to 0.38 mg/L and phosphate values ranged from 0.09 to 1.29 mg/L. The nitrate and phosphate values are higher at the wetland inlets and significantly reduced after the passage through wetlands and algal pond as elucidated in Figure 1.14.

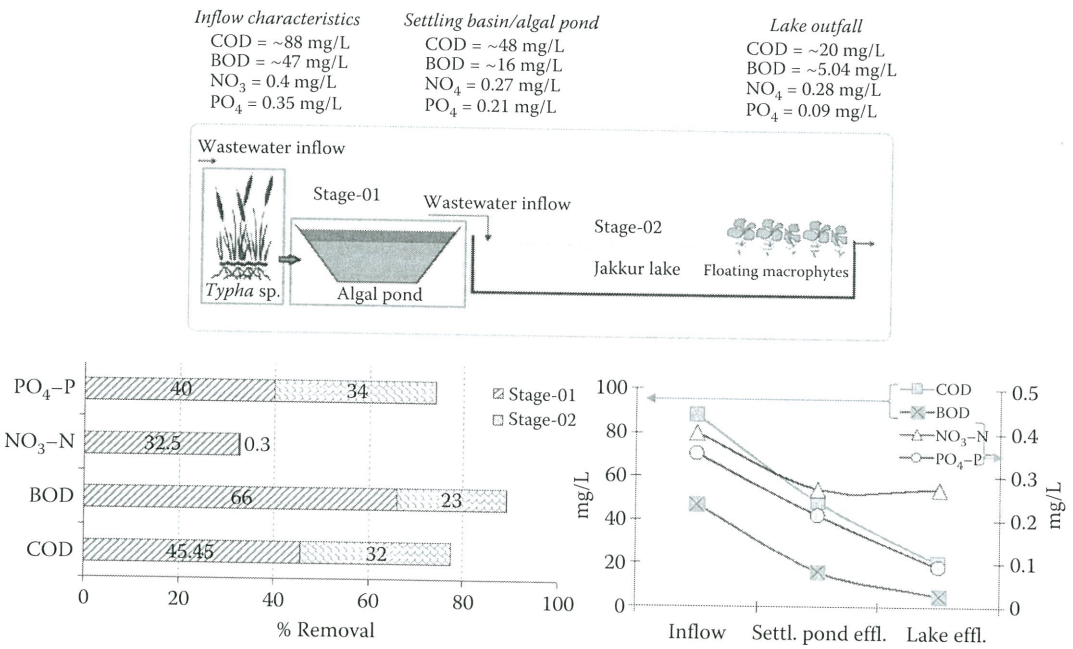

FIGURE 1.14 Integrated wastewater management system.

1.4.2.2 BOD and COD

BOD and COD are important parameters that indicate the presence of organic content. BOD is the amount of oxygen required by bacteria while stabilizing decomposable organic matter under aerobic conditions. It is required to assess the pollution of surface and groundwater where contamination occurs due to disposal of domestic and industrial effluents. COD determines the oxygen required for chemical oxidation of most organic matter and oxidizable inorganic substances with the help of strong chemical oxidant. In conjunction with the BOD, the COD test is helpful in indicating toxic conditions and the presence of biologically resistant organic substances (Sawyer and McCarty 1978). In the current study, the BOD values ranged from 17 to 128 mg/L. There was a reduction of 66% in BOD after the algal pond and 23% removal in the water which flows out of the lake. The COD values ranged from 16 to 161 mg/L. The COD reduced is by 45% in the algae pond and 32% in the lake as shown in Figure 1.14.

1.4.3 Integrated Wastewater Management System

The treatment of domestic sewage in natural systems such as constructed wetlands and lagoons is being practiced in developing nations. Significant advantages are its simple construction and operation and economic viability (Mahapatra et al. 2011a). Lagoon systems are associated with a high growth rate of beneficial phytoplankton that are caused by the influence of light and the continuous nutrient inflow. Algal growth contributes toward the treatment of wastewater by transforming dissolved nutrients into particle aggregates (biomass). Algal retention in the lagoon helps in the treatment, which has to be harvested at regular intervals to ensure effective treatment. Wetlands consisting of reed-bed and algal pond help in the removal of nutrients (Mahapatra et al. 2013a,b).

The emergent macrophytes (such as *Typha*) act as a filter in removing suspended matter and avoiding anaerobic conditions by the root zone oxidation, and the dissolved nutrients would be taken up by the lagoon algae. This type of treatment helps in augmenting the existing treatment system in complete removal of nutrients and bacteria. The combination of wetlands (with macrophyte assemblages), algal lagoon, and a sustained harvesting of algae and macrophytes would provide complete solution to wastewater treatment systems with minimal maintenance. Integrated wetland system at Jakkur provides an opportunity to assess the efficacy of treatment apart from providing insights for replicating similar systems to address the impending water scarcity in the rapidly urbanizing Bengaluru.

The treatment plant (1.6 ha) with an installed capacity of 10 MLD comprises of an Upflow Anaerobic Sludge Blanket Reactor (UASB) with an extended aeration system for sewage treatment. The treatment effluent then gets into wetlands (settling basin) of spatial extent ~4.63 ha consisting of diverse macrophytes such as *Typha* sp., *Cyperus* sp., *Ludwigia* sp., *Alternanthera* sp., *Eichhornia* sp., and so on in the shallow region (with an area of ~1.8 ha) followed by deeper algal basin (covering an area of about 2.8 ha). This being the significant functional component with macrophytes and algae jointly helps in the nutrient removal and wastewater remediation. The water from the settling basin flow passes through three sluices of which only the middle one was functional in the low flow conditions during the investigation. This water flows into Jakkur lake that spans over 45 ha. There were notably less occurrence of floating macrophytes, except near the outfalls (~0.5 ha) due to blockage of the outflow channels by solid wastes and debris. These macrophytes are being managed by local fishermen. Water in the Jakkur lake is clear with acceptable phytoplankton densities and abundant diversity indicating a healthy trophic status.

The nutrient analysis shows (illustrated in Figure 1.14) that treatment happens due to immergent macrophytes of the wetlands and algae, which removes ~45% COD, ~66% BOD, ~33% NO_3–N, and ~40% PO_4^{3-}–P. Jakkur lake treats the water and acts as the final level of treatment in the stage 2 that removes ~32% COD, ~23% BOD, ~0.3% NO_3–N, and ~34% PO_4^{3-}–P. The synergestic mechanism of STPs followed by wetlands helps in the complete removal of nutrients to acceptable levels according to CPCB norms.

Jakkur STP has been reported to treat only 6 MLD of sewage that is drawn from Yelahanka town. Yet untreated sewer channels were observed carrying voluminous wastewater into the Jakkur system alongside the treatment plant effluents. The major nutrient removal and polishing is done by the manmade wetland and the lake. This wetland comprises of emergent macrophytes such as *Typha angustata*, and so on and thus provides a key role of oxygenation in soil subsystems through root zone oxidation and entrapment of necessary nutrients that otherwise would cause an algal bloom in the lake. The algal species in this manmade wetland region (Figure 1.15) primarily comprised of members of chlorophyceae followed by cyanophyceae, euglenophyceae, and bacillariophyceae (Figure 1.16). The relative abundances are provided in the pie diagrams below.

Similarly, macrophytes play an important role in the effluent stabilization. The distribution of the macrophytes in the wetland area as well as at the outfalls of the lake is provided in Figure 1.17. *T. angustata* species were dominating (54%) in the wetland area followed by *Alternanthera philoxeroides* (28%). However, even though the macrophyte population was scarce in the lake, but still amongst them *Eichhornia crassipes* (84%) were dominating (Figure 1.18), which were only restricted to the outlet reaches provisioned by the net intervenetion to aid fishing and restrict floating macrophyte growth in the core fishing area.

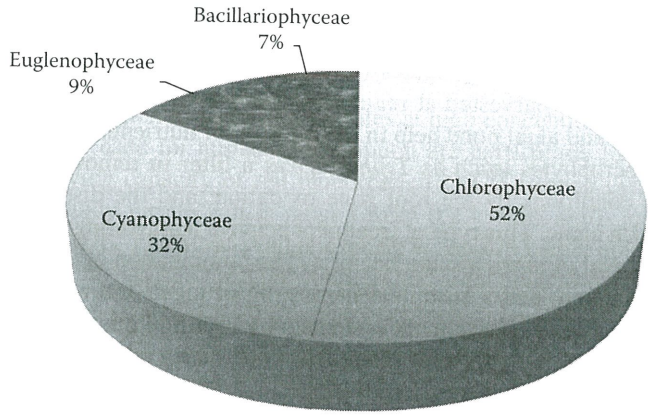

FIGURE 1.15 Composition of algae in man-made wetland system.

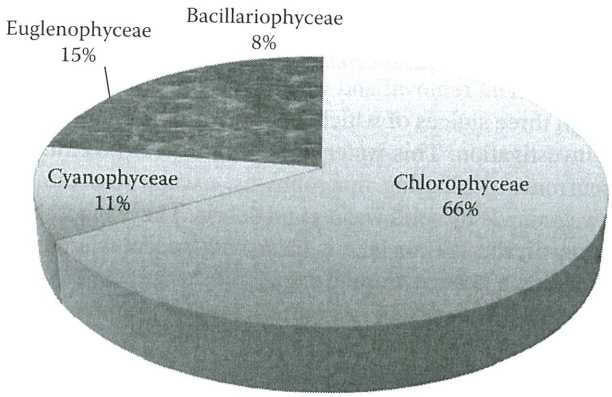

FIGURE 1.16 Composition of algae in Jakkur lake.

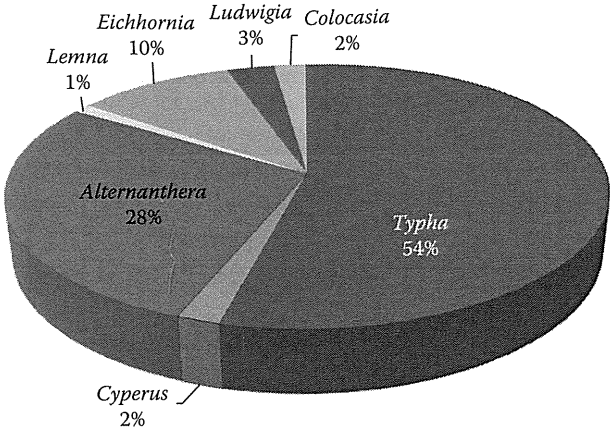

FIGURE 1.17 Composition of macrophytes in man-made wetland system.

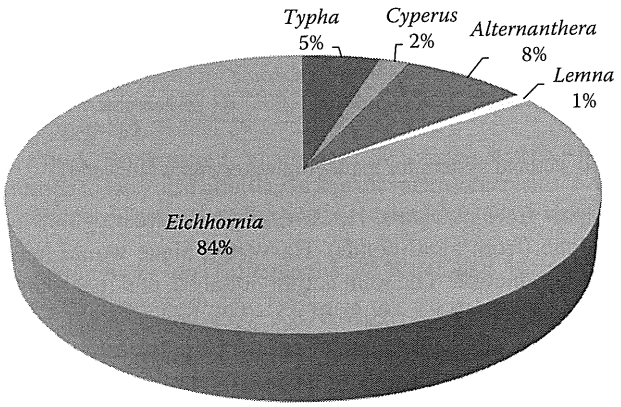

FIGURE 1.18 Composition of macrophytes in Jakkur lake.

1.4.4 Integrated Wetlands Ecosystem: Sustainable Model to Mitigate GHG Emissions

Performance assessment of an integrated wetland ecosystem at Jakkur provides vital insights toward mitigating water crisis in Bengaluru. An integrated system is outlined in Figure 1.19. The STP followed by wetland systems would help in treating water for sustainable recycle and reuse.

1.4.4.1 Functional Aspects of the Integrated Wetland Systems

Sewage treatment plant (STP): The purpose of sewage treatment is to remove contaminants (carbon and solids) from sewage to produce an environmentally safe water. The treatment based on physical, chemical, and biological processes includes three stages—primary, secondary, and tertiary. Primary treatment entails holding the sewage temporarily in a settling basin to separate solids and floatable. The settled and floating materials are filtered before discharging the remaining liquid for secondary treatment to remove dissolved and suspended biological matter. The effluents of STP are still nutrient-rich and need further treatment and stabilization for further water utilities in the vicinity.

Integration with wetlands (consisting of typha beds and algal pond) would help in the complete removal of nutrients in a cost-effective way. A nominal residence time (~5 days) would help in the

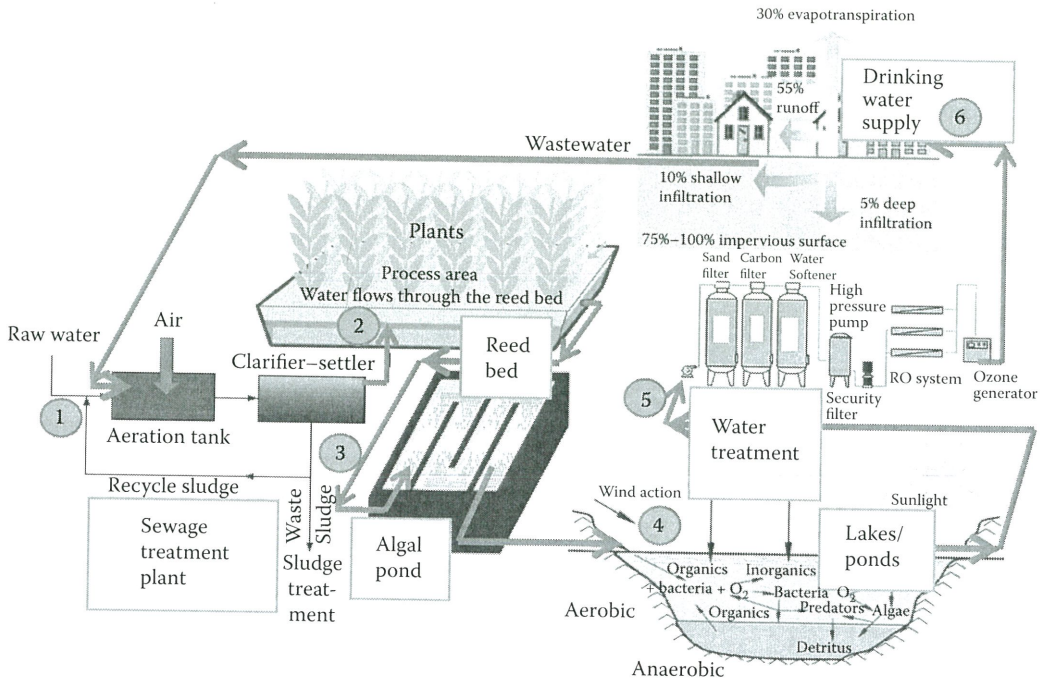

FIGURE 1.19 Integrated wetland system for managing water and wastewater.

removal of pathogens apart from nutrients. However, this requires regular maintenance of harvesting macrophytes and algae (from algal ponds). Harvested algae would have energy value, which could be used for biofuel production. The joint activity of algae and macrophytes in the wetland systems helps in the removal of 77% COD, ~90% BOD, ~33% NO_3–N, and ~75% PO_4^{3-}–P. In the case of integrated wetland systems, algae play a major role in C footprint reduction by actively uptaking the C in dissolved form (carbonic acid, bicarbonates, and carbonates) and also have the ability to take dissolved organic carbon in the dark and thus have great potential to immobilize C (up to 80% of the total input C) in the biomass.

Pilot-scale experiment in the laboratory has revealed nutrient removal of algae are 86, 90, 89, 70, and 76% for TOC, TN, Amm.–N, TP, and OP, respectively, and lipid content varied from 18 to 28.5% of dry algal biomass. Biomass productivity is of ~122 mg/L/day and lipid productivity of ~32 mg/L/day. Gas chromatography and mass spectrometry (GC–MS) analyses of the FAME showed a higher content of desirable fatty acids (biofuel properties) with major contributions from saturates such as palmitic acid [C16:0; ~40%], stearic acid [C18:0; ~34%], followed by unsaturates such as oleic acid [C18:1(9); ~10%] and linoleic acid [C18:2(9,12); ~5%]. The decomposition of algal biomass and reactor residues with calorific exothermic heat content of 123.4 J/g provides the scope for further energy derivation (Mahapatra et al. 2014).

The study reveals that surface water bodies (lakes, ponds, tanks, etc.) in Bengaluru are subjected to high nutrient loads due to the sustained inflow of untreated or partially treated sewage, altering physicochemical and biological integrity of water bodies. The treated water from STP in Jakkur still contains nutrients as primary and secondary treatments do not completely remove nutrients. However, passage of STP effluents through wetlands (consisting of emergent macrophytes and algal pond) ensures removal of nutrients to an extent ensuring potability of water. This study investigates the water quality at different stages in the integrated wetland system. The physicochemical and biological parameters were monitored as water enters the algal pond (wetland) from the STP, outlet of wetlands and at the inlet, middle, and outlets of Jakkur lake. The nutrient analysis highlights nutrient removal by wetlands due to macrophytes and algae, which removes

77% COD, ~90% BOD, ~33% NO_3–N, and ~75% PO_4^{3-}–P. The first stage comprising of emergent vegetatation and algal pond removes ~45% COD, ~66% BOD, ~33% NO_3–N, and ~40% PO_4^{3-}–P. Jakkur lake as a second stage treats the water and acts as the final level of treatment and removes ~32% COD, ~23% BOD, ~0.3% NO_3–N, and ~34% PO_4^{3-}–P. The combination of all the stages leads to a complete removal of nutrients to acceptable levels according to CPCB norms. This study provided vital insights toward an environmentally sound option of managing wastewater, while mitigating GHG emissions.

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