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Treatment efficacy of algae-based sewage treatment plants

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Abstract Lagoons have been traditionally used in India for decentralized treatment of domestic sewage. These are cost effective as they depend mainly on natural processes without any external energy inputs. This study focuses on the treatment efficiency of algae-based sewage treatment plant (STP) of 67.65 million liters per day (MLD) capacity considering the characteristics of domestic wastewater (sewage) and functioning of the treatment plant, while attempting to understand the role of algae in the treatment. STP performance was assessed by diurnal as well as periodic investigations of key water quality parameters and algal biota. STP with a residence time of 14.3 days perform moderately, which is evident from the removal of total chemical oxygen demand (COD) (60 %), filterable COD (50 %), total biochemical oxygen demand (BOD) (82 %), and filterable BOD (70 %) as sewage travels from the inlet to the outlet. Furthermore, nitrogen content showed sharp variations with total Kjeldahl nitrogen (TKN) removal of 36 %; ammonium N (NH_4-N) removal efficiency of 18 %, nitrate (NO_3-N) removal efficiency of 22 %, and nitrite (NO_2-N) removal efficiency of 57.8 %. The predominant algae are euglenoides (in facultative lagoons) and chlorophycean members (maturation ponds). The drastic decrease of particulates and suspended matter highlights heterotrophy of euglenoides in removing particulates.

Keywords Algae · Sewage treatment · *Euglena* · Facultative pond · Nutrient · Carbon capture · Biovolume

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Introduction

Domestic wastewater constitutes a major component of wastewater generated everyday in developing nations like India, Bangladesh, etc. Untreated or partially treated wastewater finds its way to the water bodies resulting in enrichment of nutrients, leading to eutrophication. Algae-based wastewater treatment plants or lagoons treat wastewater by natural oxidative processes. These treatment units consist of an anaerobic lagoon, facultative aerated lagoons followed by maturation ponds. Microbes aid in the removal of nutrients and are influenced by wind, sunlight, and other factors.

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Design of lagoons/pond systems have evolved with significant improvements during the last two decades. Investigations of hydraulic characteristics have helped in the modifications leading to more efficient ponds with aeration systems (Rinnhofer and Smith 2011; Olukanni and Ducoste 2011; Sah et al. 2012). In this context, algal systems have revolutionized the pond concepts in imparting aeration by symbiotically living with bacteria with potential nutrient uptake rates. Several plant designs for biochemical oxygen demand (BOD) removal include facultative pond, complete-mix pond systems, and anaerobic-based lagoons to stabilize large quantum of organic solids in wastewaters (Abis and Mara 2003; Mara et al. 1992, 1996; Mara 2004). Computational fluid dynamics coupled with an optimization program and field-based modeling (Olukanni and Ducoste 2011) have improved the pond design, configuration, and flow paths that avoid short circuits at a minimal cost with optimal treatment efficiencies. Among other systems, there are high-performance aerated pond systems, duckweed pond system, N removal pond systems, and modified high-performance aerated lagoon systems for simultaneous nitrification and denitrification (Oswald 1990; Mara et al. 1996; Zimmo et al. 2003; Valero et al. 2010; Sah et al. 2012).

Waste stabilization ponds exploit symbiotic relationships between algae and bacteria in the removal of nutrients. In this regard, algae, the primary producers, generate O_2 (during photosynthesis) that aid in the efficient oxidation of organic matter with the help of chemoorganotrophic bacteria. Type and diversity of the algae grown are potential indicators of treatment process (Amengual-Morro et al. 2012). The bacterial system disintegrates and degrades the organic matter providing the algae with an enriched supply of CO_2, minerals, and nutrients. The stabilization pond-based treatment system under investigation at Mysore depends largely on the algal–bacterial symbiosis for its treatment along with substantial odor reduction. The treatment plants are with a basic design comprising of facultative aerated lagoons followed by maturation ponds. During the investigation period, aerators were kept nonoperational at Vidyaranyapuram treatment plant in Mysore to essentially assess the efficacy of algae-based treatment options. Fermentative bacteria were being used for rapid conversion of organic matter to easily reducible substrates. The quantity of sewage generated in this catchment is about 150 million liters per day (MLD) and about 65 % (95 MLD) is being treated, while the balance is tapped by farmers for irrigation. The wastewaters, before entering the treatment farm, are fed with fermentative-bacterial inoculums (OS-I and II).

The function of algae in ponds and its mechanism in enhancing the treatment efficiencies have seldom been addressed in sewage treatment system analysis and most of the earlier studies (Jamwal et al. 2009) have focused on the efficiencies of treatment plant only. The objective of the present study is to investigate the algal dynamics in terms of abundance, distribution, cellular characteristics, biovolume, carbon uptake efficiency, and biomass densities for the possible uptake and assimilation of nutrients and analysis of the treatment efficiency of the sewage treatment plant (STP) at Vidyaranyapuram.

Material and method

Mysore with the elevation of 650 m (meters above mean sea level) lies between 12°9′–11°6′ N and 77°7′–76°4′ E (Fig. 1a) in Karnataka. The region is a historic princely province with a current population of about 0.983 million (in 2011) compared to 0.795 million in 2001. The temperature ranges from 14 to 34 °C and the monsoon showers start from the end of May and continue till October. Prominent lakes of the city are Kukkarahalli, Karanji, and Lingambudhi. Domestic water requirements in the city are met mainly by Cauvery and Kabini rivers and partly by groundwater sources (at outskirts).

Study area

The wastewater generated in Mysore city by virtue of gravity finds its way into three valleys. Northern drainage system leads to 30.0 MLD STP (mechanical system with aerators) at Kesare Village, Mysore. The southwestern drainage flow connects to 67.65 MLD Vidyaranyapuram STP (biological treatment that uses essential microorganisms (OS-1 and 2) inoculum) before entering Dalvai kere. Further south is the 60 MLD Rayankere STP (remediation based on Fermenta's bacillus) at H. D. Kote Road. The field investigations have been carried out at Vidyaranyapura STP, located between 12.273681–12.270031° N and 76.650737–76.655947° E, adjacent to solid waste landfill site at

Fig. 1 **a** Sewage treatment plant at Vidyaranyapura. **b** Inflow and outflow parameters at Vidyaranyapuram STP, Mysore. Note: sampling locations are marked: *1* inlet to *7* outlet

the foothills of Chamundi Hills in the catchment slope of the feeder to Dalvai Lake. The STP consists of two facultative lagoons (each of 5.05 ha of area and 3.5 m depth) and two maturation ponds (each of 2.5 ha of area and 1.5 m depth). Table 1 lists the details. The treated water traverses about 20 km via the Dalvai kere and finally, reaches Kabini River, which is a drinking water source. This necessitates the investigation of the STP's treated effluent to ensure noncontamination of Kabini water. The residence time is computed as per Eq. 1, considering the flow as laminar with parameters such as volume (of a lagoon) and mean flow:

$$Q = V/t \tag{1}$$

where Q = mean flow of wastewater (million liters per day);
V = lagoon volume (liters); and
t = residence time in days.

The mean residence time of wastewater is 11.8 and 2.5 days, respectively, at the facultative and maturation ponds.

Assessment of treatment efficiency

Treatment efficiency of STP was assessed based on regular monitoring (seasons) carried out during 2010–2011. Samples of the raw influent [1] at various points [2, 3, 4, 5, and 6] of facultative ponds and maturation ponds [7] were collected during the monsoon and the summer seasons (Fig. 1b). Parameters such as dissolved oxygen (DO), pH, electrical conductivity (EC), oxidation reduction potential (ORP), and turbidity were measured on the site. Samples were also analyzed for chemical oxygen demand (COD) on the spot. Samples were collected in disinfected plastic containers (21) and were brought to the laboratory for further analysis. Various organic and inorganic analyses

Table 1 Site specifications with residence time at Vidyaranyapuram STP

Lagoon	Length (m)	Width (m)	Depth (m)	Surface (m^2)	Volume (m^3)	Detention time (days)
Facultative lagoon (2)	312	162	3.5	50,544	176,904	11.8
Maturation pond (2)	172	145	1.5	24,940	37,410	2.5
						14.3

were performed as per American Public Health Association protocol (APHA 1992). Samples were tested for filterable and nonfilterable BOD, COD, total Kjeldahl nitrogen (TKN), Amm-N (NH_4-N), nitrite-N (NO_2-N), nitrate-N (NO_3-N), orthophosphates (OP), and total phosphates (TP) with other physicochemical parameters.

Computation of algal productivity

Algal samples collected from sampling locations were identified using standard keys (Prescott 1973, 1982) based on their external appearance, color, morphological characteristics, size, habitat, structure and orientation of chloroplast, cellular structure and pigments, etc. Wastewater samples collected were concentrated by centrifuging 15 ml volume. Algae were enumerated using three replicates of 20 μl of the concentrated sample, where it was placed over the slides with cover slips for microscopic observations and density was computed by Eq. 2 that determines the ratio of number of cells counted in the given quantity of water sample:

$$\text{Number of algal cells/ml} = \frac{\text{Number of organisms counted}}{\text{Quantity of water sample in a slide.}} \quad (2)$$

Algal samples were observed under a microscope and algal biovolume was calculated that was later equated with algal weight. Samples collected from the field were dried for computing the total dry weight. Quantification for unit area per volume was performed by taking 10-l volume for algal samples and the temporal algal productivity was estimated with periodic assessment of dry weight and volume of water (Hillebrand et al. 1999).

Data analysis

Nonparametric Spearman's rank correlation coefficient (r) is computed to explore significant relationships between changes in physicochemical variables against biological variables (relative abundance of algal species). Multivariate analysis (Martin-Cereceda et al. 2002)—canonical correspondence analysis (CCA) was performed to understand transitions in biological communities with varying physicochemical variables and to know relationships among them. Higher variability in abundance was tackled by $\log_{10}$ transformation of the values. Bray–Curtis cluster analysis (CA) was performed in order to find out the spatial similarity and patterns across sites. These statistical analyses were carried out using open source statistical package PAST 2.14 (downloaded from http://www.nhm.uio.no/norlex/past/download.html).

Results and discussion

Physicochemical analysis

Physicochemical parameters analyzed are presented in Table 2.

pH

pH is an important parameter for evaluating treatment plant efficiency and water quality and is provided in Table 2. The pH value ranged from 6.78 at the inflows to 8.68 at the facultative ponds, which was attributed to acidogenic phase in the inflow waters (Kayombo et al. 2002) and higher photosynthesis due to prolific algal growth in ponds (Lai and Lam 1997; Veeresh et al. 2009). Experiments conducted in large-scale high rate algal ponds (HRAP) also showed a pH~9 (Craggs et al. 2012a). The high pH values are attributed to higher photosynthetic rates of algae, drawing more dissolved CO_2 from the waters and thereby causing high bicarbonate and carbonate concentrations, known as alkalinity. High carbonates cause calcium and magnesium ions to form insoluble minerals, leaving sodium as the dominant ion in solution.

Biochemical oxygen demand

Unfiltered BOD in the raw wastewater ranged from 55 to 307 mg/l, while filtered BOD ranged between 29 and 96 mg/l during the dry season (Table 2). The particulate BOD in inflows was mostly contributed by food and excretory wastes as evidenced by the brownish gray color of the incoming wastewater. The decrease of the filtered and the unfiltered BOD from the inflows to the facultative pond to almost 60 % during the detention period of 36 h illustrates the functioning of anaerobic zone of the lagoon. In a study conducted by Faleschini et al. (2012), 41 % of unfiltered BOD removal happened due to sedimentation in the first segment of the FP and ~55–67 % of unfiltered and 81–90 % of filtered BOD removal were observed during their study period. After the first point (TP-01,

Table 2 Physicochemical characteristics of facultative and maturation ponds

Parameters	Comm. inflow	FP (anoxic)	FP-01	FP-02	FP-03	FP outlet	MP outlet
Sampling site	**[1]**	**[2]**	**[3]**	**[4]**	**[5]**	**[6]**	**[7]**
pH	6.78	6.91	7.35	7.78	8.68	8.34	7.74
Temperature (°C)	27.6	27.5	28	28.1	29.6	31.3	28.9
Salinity (mg/l)	486	572	573	566	535	534	565
Total diss. solids (mg/l)	782	920	925	968	861	859	904
Total sus. solids (mg/l)	460	288	224	389	170	94	32
Elec. cond. (μS/cm)	978	1,152	1,159	1,138	1,080	1,082	1,142
Dissolved oxygen (mg/l)	0	0.88	3.6	5.8	12.9	7.43	4.54
COD (mg/l)	458.7	224.0	213.3	362.7	202.7	208.0	96.0
Filterable COD (mg/l)	160.0	74.7	106.7	109.3	98.7	80.0	85.3
BOD (mg/l)	307.0	112.2	91.8	224.0	100.0	78.6	55.1
Filterable BOD (mg/l)	96.9	39.8	31.6	36.2	34.7	27.6	29.6
Nitrates (mg/l)	0.202	0.203	0.182	0.181	0.166	0.171	0.156
Nitrites (mg/l)	0.015	0.013	0.021	0.014	0.007	0.057	0.006
TKN (mg/l)	47.04	40.32	33.60	43.68	31.08	21.84	30.24
Ammonia (mg/l)	25.67	25.44	21.35	20.54	20.73	20.99	23.78
Phosphates (mg/l)	6.08	4.13	6.03	6.75	6.30	5.94	2.37
Total phosphates (mg/l)	7.36	7.61	10.42	7.81	10.76	10.81	10.12
Alkalinity (mg/l)	480	520	480	560	500	520	480
Carbonates (mg/l)	0	40	40	40	40	80	120
Bicarbonates (mg/l)	480	480	440	520	460	440	360
Ca (mg/l)	56	59	55	56	63	59	58
Mg (mg/l)	26	32	30	36	31	33	32
Tot. hardness (mg/l)	248	280	260	288	284	284	276
Na (mg/l)	153	200	186	208	204	201	192
K (mg/l)	37	40	39	44	42	40	41
Chlorides (mg/l)	99	116	114	122	119	119	116
ORP (mV)	−135	−16	59	58	45	37	35

Sampling sites are given in bold letters (refer Fig. 1 for the location details)

Fig. 2), there was almost no variation in BOD, except at point FP-02, where the unfiltered BOD shoots up to 224 mg/l. However, the filtered BOD values are almost the same compared to earlier values. The increase in BOD (Fig. 2) is due to the proliferative growth of euglenophycean members. BOD values up to 100 mg/l are the permissible limit for irrigation use (CPCB n.d, India). The algal count 9.3×10^5 cells/ml and 2.4×10^5 cells/ml were observed in facultative ponds and maturation ponds, respectively, during midday.

The algal members contribute to the particulate BOD in the effluent, which is evident from the surface BOD loading of about 60 % in anaerobic zone that extends up to 10,000 m^2 (Valero et al. 2010; Faleschini et al. 2012). Given the adequate anaerobic environment, the BOD removal could be as high as 92 % in

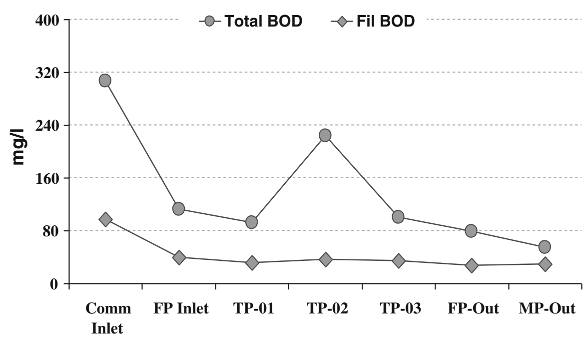

Fig. 2 Variation of filterable and total BOD with residence time

integrated waste stabilization ponds (Tadesse et al. 2004), 96 % in facultative lagoons (Jamwal et al. 2009), and 82–91 % in HRAP (Craggs et al. 2012a).

Chemical oxygen demand

COD infers to the oxygen equivalent of the organic material in wastewater that can be oxidized chemically. COD of the unfiltered raw influent and filtered effluent were 458 and 160 mg/l, respectively, and were similar to BOD trend (Table 2). The maximum surface COD removal of 50 % happened in the initial part of the facultative lagoon (in the anaerobic zone). The net unfiltered COD removal efficiency of the STP was around 80 % and filtered COD removal efficiency was around 50 % contrary to COD removal of 63 % (Kayombo et al. 2002), 90 % (Tadesse et al. 2004), and 92 % (Jamwal et al. 2009). Maturation ponds showed a slightly higher filtered COD value than the facultative lagoons that can be attributed to some external interference that provided additional organic loads. Higher algal density at the middle of the ponds has contributed to a higher particulate COD value (Fig. 3).

Major COD removal mechanism is sedimentation of solids that settle (as in septic tanks) and subsequent anaerobic digestion in the resulting sludge layer. Bacterial groups involved are the anaerobic acidogens indicated by lower pH (~6) values near the anaerobic zones and methanogens. Around 30–50 % of the influent BOD leaves the facultative pond in the form of methane (El-Fadel and Masood 2001). The remaining nonsettleable BOD is oxidized by the normal heterotrophic bacteria such as *Pseudomonas* sp., *Flavobacterium* sp., *Alcaligenes* sp., etc. Bacteria use oxygen produced during the photosynthetic activities of the microalgae that grow naturally and profusely in facultative ponds. The algal–bacterial activities result in a high proportion of the BOD that ends up as algal cells and does not escape as methane and carbon dioxide in the pond. Thus, in facultative ponds, sewage COD is primarily converted into algal COD.

The correlation between COD and BOD indicates readily degradable organic matter contrary to slowly biodegradable suspended solids (SS) as in complex waste effluents containing refractory substances (Eckenfelder 1989). The average influent and effluent COD/BOD5 ratios for the treatment plants were calculated and it is observed that the COD/BOD5 ratio frequently varied for influents and effluents (Fig. 4). A ratio ranging from 1.49 to 1.87 for the influent wastewaters are comparable to the earlier studies (Metcalf and Eddy 2003). The typical COD/BOD5 ratio of domestic wastewaters is usually in the range of 1.25 to 2.5. However, for treated effluents, it ranged from 2.02 to 2.9 indicating a relatively higher proportion of the non-biodegradable content in treated effluent than raw wastewater.

There is a marked transition of the color and nature of the wastewater as it passes through the various stages of the treatment ponds system. Initially, the sewage color is very dark, either black or gray, as shown in Fig. 5a. After the SS reduction and transformation of the particulate organic matter into inorganic nutrient forms, the algae (green color) proliferates in these facultative regions as depicted in Fig. 5b and finally, when the wastewater reaches the final maturation ponds, it is oxidized and looks clear as illustrated in Fig. 5c. Table 3 summarizes earlier investigations of the treatment efficiencies of facultative pond based on sewage treatment systems with various process parameters and percentage removal efficiencies of nutrients.

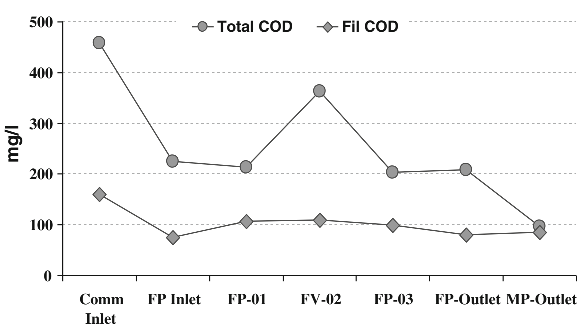

Fig. 3 Variation of filterable and unfiltered COD with residence time

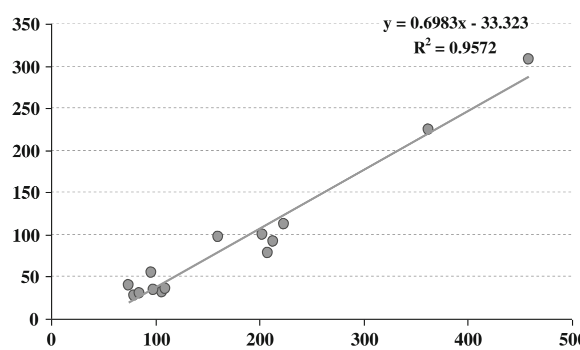

Fig. 4 Correlations between BOD and COD

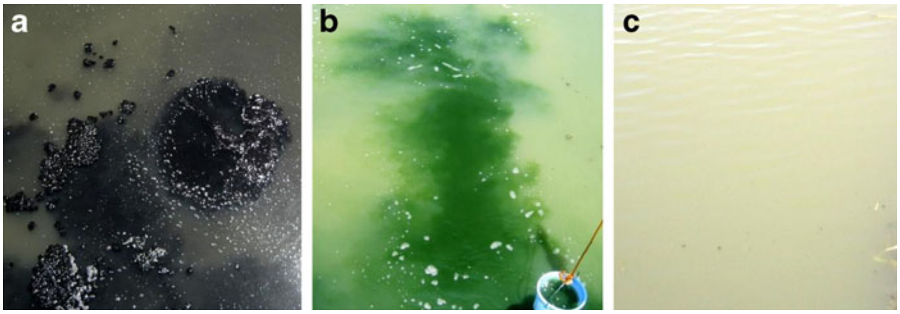

Fig. 5 **a** Near FP inflow (anaerobic zone—sludge upwelling). **b** Facultative zone (high algal growth—euglenophyceae) and **c** maturation pond—water is clear with slightly muddy color

Inorganic carbon

Carbonates are one of the most important dissolved components of wastewaters. The amount of carbonates controls the diffusion of carbon dioxide while governing water pH and buffering. Very little carbonate (10 mg/l) enters at inlet to the raw wastewater and undergoes various stages of treatment. Progressive increase in the amount of carbonates was observed, which was highest at the outfalls of the facultative ponds and maturation ponds (Table 2), which was attributable to higher pH due to fast algal photosynthetic activity (Lai and Lam 1997). However, the bicarbonate concentrations increased at the middle regions of the facultative ponds and declined in the final maturation ponds.

Suspended solids

SS range from 460 mg/l (at inlets) to 32 mg/l. Higher SS at inlets are due to semidecomposed and decomposed solids from food, etc. There was a gradual reduction of SS at the middle and the effluents of the facultative lagoon. SS of 389 mg/l at the middle regions is due to the dense suspended algal solids. About 80 % turbidity is removed in the maturation ponds that have minimum SS (32 mg/l). The SS removal efficiency is about 93 %, which is comparable

Table 3 Comparative account of treatment efficiencies of facultative pond systems with their percentage removal efficiencies of earlier studies

Type of WSP and source	Depth (m)	S. loading (kg/ha day)	Detention time (day)	Temp. (°C)	TSS rem (%)	COD rem (%)	BOD rem. (%)	Amm-N rem (%)	N rem (%)	P rem (%)
FP, Karchanawong and Sanjitt (1995)	1–1.1	16–160	1.6–16	20–26	−4 to −100	3–33	22–44	20–72	4–55	20–22
SP, Karchanawong and Sanjitt (1995)	1–1.1	16–160	1.6–16	20–26	36–90	34–68	44–72	5–79	4–75	32–90
WSP, Veenstra et al. (1995)	1.5–2	250–340	15	20	90	78.75	87.5	2–25	–	–
AIWP, Green et al. (1995)	2	–	2–3	20	25	–	56	29	72	19
WSP, Ceballos et al. (1995)	2.2	296	61	25–27	67	–	95	39	45	8
FP Aerated biofilter, Goncalves and de Oliveira (1996)	1.8	NG	NG	NG	25–90	63	NG	86	67	–
WSP, Kayombo, et al. (2002)	1.6–1.9	155–695	3–6	23–28	–	66.3	–	–	–	–
AIWP, Tadesse et al. (2004)	1.2–3.5	210–5,560	4–8	15–30	89–94	90–97	92–99	42–87	–	42–94
FP, Jamwal, et al. (2009)	–	–	18	10–33	98.12	92.31	96.47	–	86.3	–
WSP, Veeresh et al. (2010)	1.2	–	8–10	24–31	–	–	79	–	–	69[a]
HRAP, Craggs et al. (2012b)	0.35	–	–	8–20	–	–	87	51	–	19
FP, Faleschini et al. (2012)	1.5	55–68	24–31	8–20	90	–	79–90	90	–	–
FP, present study (2012)	1.5–4	400–900	12–14	21–26	93	79	82	6	36	61[a]

FP facultative pond, *SP* spinach pond, *WSP* waste stabilization pond, *AIWP* advanced integrated wastewater pond, *HRAP* high rate algal pond

[a] Orthophosphates

to 90 % (Veenstra et al. 1995) and 89–94 % (Tadesse et al. 2004) removal.

Nitrogen removal

In anaerobic regions, organic nitrogen is hydrolyzed to ammonia. Higher ammonia values at the inflows is due to higher residence time in the sewer allowing most of the organic nitrogen being converted into ammonia before reaching the STP. Nitrogen is incorporated into new algal and bacterial biomass by biosynthesis. Eventually, cell growth declines and they settle at the bottom of the pond; around 20 % of the biomass is nonbiodegradable and the nitrogen associated with this fraction remains immobilized in the pond sediment (Mahapatra et al. 2011b). Nitrogen associated with the biodegradable fraction eventually diffuses back in the water, which is recycled back into biomass and the process repeats (Mahapatra et al. 2011a). At high pH levels (pH>9), some of the ammonia leaves the pond by volatilization (Zimmo et al. 2003).

Nitrate nitrogen (NO_3-N) is a necessary primary macronutrient for the biota in aerated treatment systems. The raw influent sewage has NO_3-N of 0.202 mg/l (Fig. 6) and a significant amount of it was not detected as a result of low nitrification but most of it was in the form of ammonia. NO_3-N in the effluent of the facultative pond and the maturation ponds were 0.171 and 0.156 mg/l, respectively (Table 2). Earlier studies have reported nitrate concentrations >10 mg/l at higher organic loads with competitive algal activities (Faleschini et al. 2012). Nitrifiers having slow growth rates will only be of significance in the water column of systems with long retention times (Pauer and Auer 2000). The populations of nitrifying bacteria are relatively low in wastewater stabilization pond (WSP) due to the absence of physical attachment sites in the aerobic zone and also by algal inhibition.

Inhibition of nitrification is dependent on the quantum of organic carbon due to ammonification rates and high concentrations of ammonia (Strauss and Lamberti. 2000). Excessive ammonia causes the decoupling of the two nitrification steps by selectively inhibiting nitrite nitrogen (NO_2-N) oxidation, leading to NO_2-N accumulation, which leads to positive feedback resulting in further eutrophication (Chen et al. 2010).

Nitrite nitrogen (NO_2-N) ranged from 6.3 to 21.1 μg/l (Table 2) and was significantly less compared to other forms of N (ammonia and nitrates). The influent raw sewage had a value of 14.9 μg/l of NO_3-N (Fig. 6) and the highest values were recorded in the initial portion of the facultative lagoons. A sudden increase in the nitrite values at this region may be because of incomplete nitrification, while maturation pond effluents showed the lowest values (6.3 μg/l).

Ammonia nitrogen (NH_4-N) values, elucidated in Fig. 6, ranges from 20.54 to 25.67 mg/l and were higher compared to the other forms of N (Table 2). The influent raw sewage values were around 26 mg/l, while effluent ammonia values were around 23.78 mg/l. The trend shows that there is a drop in the NH_4-N present in anaerobic zone of the facultative lagoon due to settling of particulate organic matter and consequent higher microbial activities, which is similar to an earlier report (Faleschini et al. 2012). There are possibilities of NH_4-N removal by methanotrophs that support nitrification within the sludge under anoxic conditions and consequent denitrification in the wastewaters (Valero et al. 2010). Studies conducted in hectare-scale

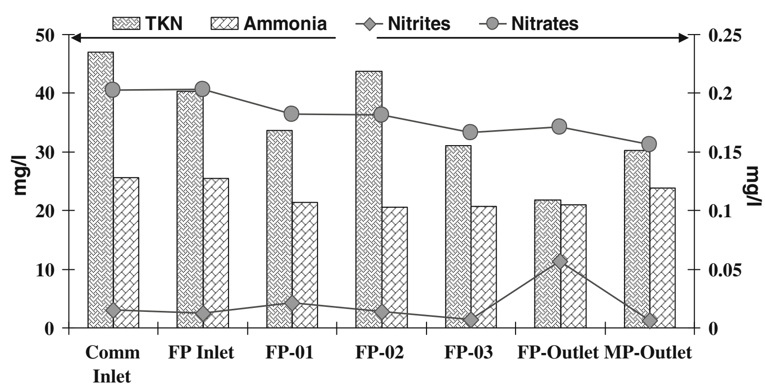

Fig. 6 Variations of N species at facultative ponds and the maturation ponds

HRAP have reported 64–67 % removal of NH_4-N (Craggs et al. 2012a).

The increase in NH_4-N values from the outfall of the facultative pond to the outfall/effluent of the maturation ponds is due to seepage of waste from other sources or N waste products of the native biota of the ponds. NH_4-N volatilization is proposed as the major sink for N in anaerobic/anoxic systems with high NH_4^+ influents like partially treated or untreated sewage (Kalff 2002; Bolan et al. 2004) in contrast to other studies (Zimmo et al. 2003) that showed NH_4-N volatilization losses to be <1.1 % compared to the other N losses. The experimental results of N speciation in relation to pH also suggested against NH_4-N volatilization in ponds (Faleschini et al. 2012).

TKN (~48 mg/l) values were higher near the inflows and lower (~22 mg/l) at the outlets of the facultative lagoons (Table 2). Outfalls of the maturation ponds showed comparatively higher TKN (Fig. 6) due to higher activity of zooplanktons. This is similar to 86 % TKN removal in oxidation ponds at Delhi (Jamwal et al. 2009).

Phosphate removal

Phosphates are the key and limiting nutrients deciding the system's productivity in terms of biomass, necessitating P regulations in STP's. OP were higher at the middle portions of the facultative pond, indicating active mineralization with higher algal biomass (6.75 mg/l). Lowest values of OP were found in the outfalls of the maturation ponds (2.37 mg/l) but TP were higher (Table 2, Fig. 7) in the aerated regions of the facultative pond and the maturation ponds (10.12–10.76 mg/l). This could be attributed to the higher algal biomass and, therefore, higher organic P. Studies conducted on HRAP showed a mere OP removal of 14–20 % (Craggs et al. 2012a). The lowest TP values were observed at the raw influent inflow regions owing to partial mineralization as influent sewage reached the STP, thus showing a higher value of OP in the inlets. P removal reported earlier varied from 19 % (Green et al. 1995) to 21 % (Karchanawong and Sanjitt 1995) and 68 % in advanced integrated wastewater pond (AIWP), (Tadesse et al. 2004).

Algal dynamics in ponds

Algae are the key players in oxygenation in pond-based systems. The algal solids produced during the treatment process are potentially useful harvestable biomass that can be used as fertilizers and food and raw material for biotechnological industries (Pearson et al. 1987). Sensitivity of the algal biomass to varied organic nutrients affects growth, productivity, and efficiency of the treatment systems. Therefore, the type of the species present and the quantity of the biomass are potential indicators of the plant efficiency.

Facultative ponds

The quantum of algae growing in the facultative pond is a function of the surface organic loading. Species diversity decreases with the higher organic loadings (Konig 1984; Konig et al. 1987) as evident from fewer algal genera (flagellate genera, i.e., euglenoides) at the facultative ponds compared to that of maturation ponds with predominating nonflagellates as *Micracitinium* sp. and *Chlorella* sp. (Table 4), revealing the functional aspects of the treatment systems. Similar to the earlier reports (Veeresh et al. 2009; Amengual-Morro et al. 2012),

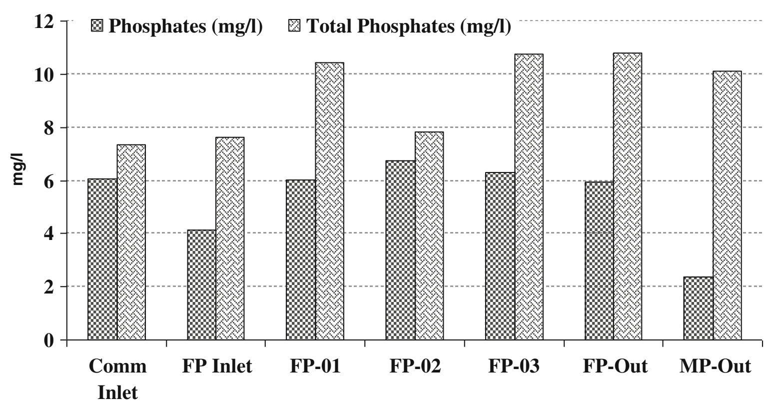

Fig. 7 Variations of P species at facultative ponds and the maturation ponds

Table 4 Relative abundance of microalgal species in STP (% of 500 counts)

Algal genus	Inlet	FP	FP outlet	Maturation pond
Chlorophyceae				
Micracitium pusillum	–	–	–	19.5
Monoraphidium graffiti	–	20	2	6.5
Monoraphidium contortum	–	–	–	3.5
Chlorococcum sp.	2	–	–	3.25
Chlorella vulgaris	–	–	–	24.5
Botrycoccus brauni	–	–	–	2
Chlorella ellipsoidea	–	–	–	7.25
Aataerococcus sp.	–	–	–	0.5
Eudorina sp.	–	–	–	0.5
Selenastrum minutum	–	–	–	2
Pyrobotrys gracilis	–	–	–	0.5
Pandorina morum	–	–	–	0.5
Cyanophyceae				
Chroococcus disperses	–	–	1	3
Planktothrix sp.	82	–	–	10.25
Phormidium sp.	–	–	–	0.5
Chroococcus limneticus	–	–	–	1.25
Oscillatoria sp.	–	–	–	0.75
Planktolyngbya sp.	–	–	–	0.5
Gomphosphaeria sp.	–	–	–	0.25
Euglenophyceae				
Euglena sp.	5	35	40	2
Lepocinclis sp.	–	15	–	0.5
Euglena aqus	–	–	–	1.5
Phacus chloroplasts	–	–	–	0.25
Trachelomonas sp.		40	57	–
Bacillariophyceae				
Nitszchia sp.	1.25	–	–	10
Cyclotella sp.	1	–	–	–

dominance of *Euglena* sp. were observed in the facultative ponds.

At the initial reaches, where the organic matter was high, there were fewer algal genera as algal biomass decreases with increased BOD surface loadings in facultative ponds (Figs. 2 and 5a). Pure population of flagellate euglenoides (*Euglena* sp.) was noticed at higher organic loads (400–900 kg BOD5/ha/day). Thirty-four algal genera were observed in conditions where the light intensities remained relatively constant. Euglenophytes were the dominant members in facultative ponds contrary to earlier studies that reported the dominance of chlorophytes (Bernal et al. 2008). At 770 kg BOD5/ha/day, *Chlamydomonas*, *Pyrobotrys*, *Phacus*, and *Euglena* sp. were among the most resistant algae to such conditions (Athayde 2001). The maturation pond had an organic loading of ~200 kg BOD5/ha/day, where *Micracitinum* sp. and *Chlorella* sp. dominated in contrast to the reports of abundance of *Ankistrodesmus* sp. and *Scenedesmus* sp. at surface organic loadings below 75 and 50 kg BOD5/ha/day (Athayde 2001). In the recent study, cyanophycean cells (*Spirulina* sp. and *Microcystis aeruginosa*) were reported to be dominant during the nutrient-limiting regimes, when the organic matter load was <20 m^3/day in wastewater flows. However, the chlorophycrean members (*Chlamydomonas* sp., *Oocystis* sp., *Pandorina morum*, *Eudorina elegans*, and *Scenedesmus* sp.) dominated during the nutrient-rich inflow periods in the stabilization ponds (Amengual-Morro et al. 2012)

Prevalence of higher concentrations of ammonia throughout the system is indicative of higher organic loading that might control/check the growth of sensitive algal species. Ammonia and sulfate inhibitions were reported in earlier studies (Konig 1984; Konig et al. 1987; Mills 1987; Pearson et al. 1987; Athayde 2001). It has been also observed that genera like *Chlorella* grow in a wide range of organic loading. Studies show its preference for lower organic loads (28 kg BOD5/haday) in maturation ponds (Athayde 2001). The present study reveals that the changes in cell concentration of euglenoides in facultative pond and *Micracitinium* sp. in maturation ponds can be used as the indicator to forecast changes in loading conditions in pond systems.

Total algal biomass as determined by algal microscopic counting and enumeration is higher in facultative ponds than in the subsequent maturation pond. This indicates the reduction in available nutrients and also the increased grazing pressures by the zooplankton population under aerobic conditions in maturation ponds. Several algal genera common in facultative ponds, including *Euglena* sp., are capable of heterotrophic growth on organic substrates such as acetate released as a result of anaerobic degradation of organic material in the anaerobic sediments (Mills 1987; Pearson et al. 1987). *Euglena* species have been observed to migrate to lower anaerobic organic-rich sediments in facultative ponds at night (Konig 1984). However, the significance of this in terms of overall organic carbon removal from ponds is not clear and needs further studies.

Anaerobic zone

The anaerobic part of the ponds, especially the initial regions, receives larger quantity of total maximum daily load (TMDL). During the field survey, gray color was observed in the anaerobic zone of the facultative pond, which is attributed to a balanced loading. However, the sludge upwelling at the initial anaerobic reaches was consistently observed during the field investigation. This would have resulted in impaired facultative pond performance. These regions showed lower algal cell counts and were mostly dominated by the filamentous cyanophycean members such as *Plakntothrix* sp. and *Planktolyngbya* sp. and very few flagellates.

Facultative zone

Higher euglenoid densities (~10^6 cells/l) were found in the facultative ponds. There was a significant fluctuation in the algal densities ($p<0.05$) across the three different travel paths, viz. sites 3, 4, and 5. These fluctuations were not correlated with temporal variations. These differences in the algal densities are attributed to their abilities to sustain at higher carbon loads (through heterotrophy). The difference in the surface BOD loading can be one of the possible reasons for the diversity in algae.

Differences in the spatial and temporal distributions of algal counts have been attributed to the stratification of algae during daylight hours into a distinct movable band (40–50 cm wide), which corresponded to the water column in the middle reaches of the facultative pond. These algal bands were carefully observed and it was found that these bands move up and down through the water in response to changes in sunlight intensity and associated wind movement. When it approaches the effluent take-off level, a very high concentration of algae appears in the effluent (making the water color dark green). As it moves towards the maturation pond, the concentration drops. The vertical movement of algae varies with the time of the day and according to the wind movement. Present observations confirmed that the algae communities in these ponds were the motile euglenoides and they move on to the bottom sediments of the pond at night. Algal species composition (dominance and community structure) can be an essential tool, which can indicate pond type and BOD surface loading. In the present investigation, it was observed that the euglenoides predominate the facultative ponds. During the study, the facultative pond was essentially comprised of flagellate algal genera (*Euglena* sp., *Lepocinclis* sp., *Phacus* sp., *Pyrobotrys* sp., *Trachelomonas* sp., and *Chlamydodmonas* sp.). However, the nonmotile algae (*Micractinium* sp., *Scenedesmus* sp., and *Chlorella* sp.) dominate the maturation ponds as given in Table 4 (Fig. 8d). The diversity of algal species increases with improved effluent quality. The present study indicated four to five species existing in

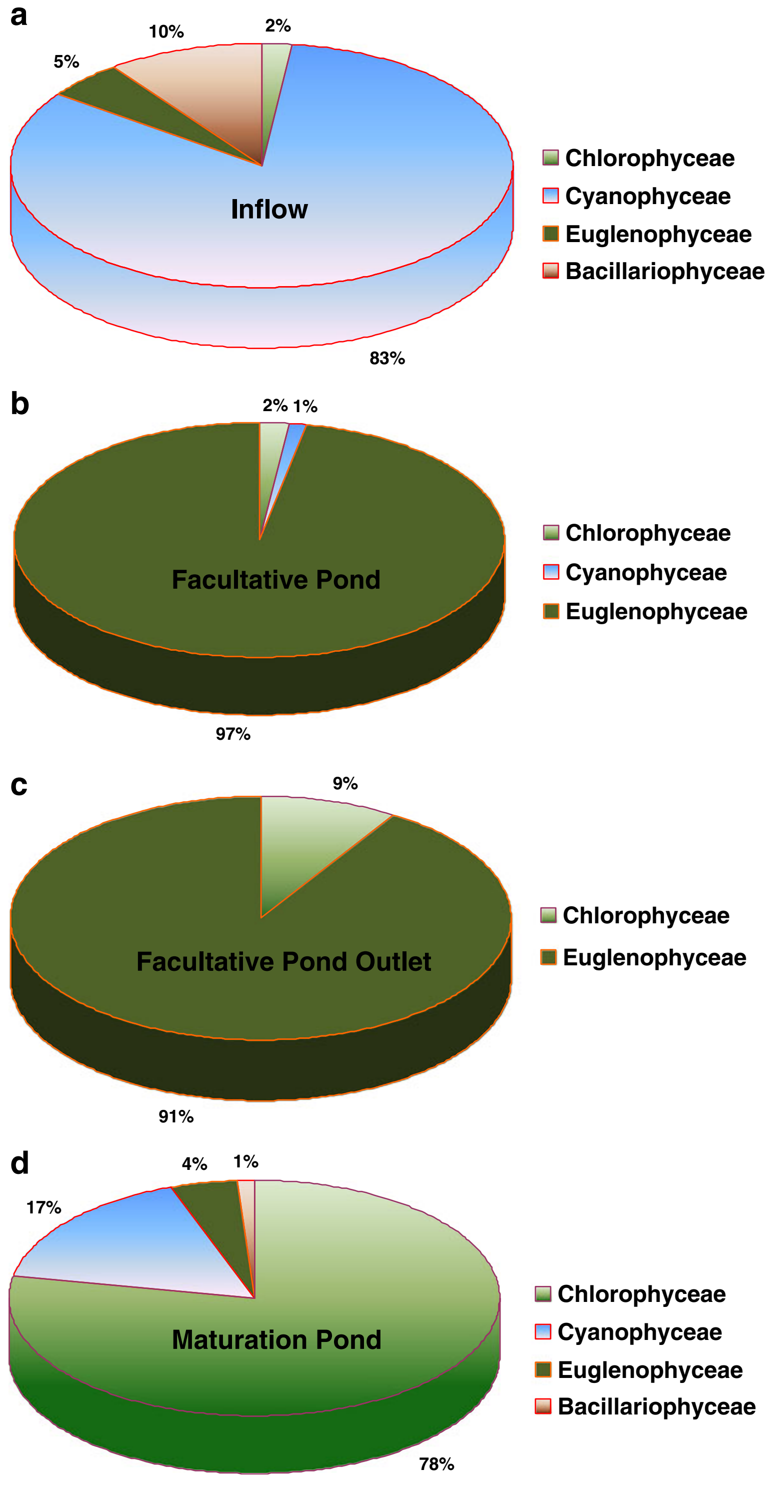

Fig. 8 **a** Algal distributions in the inflow raw feed (sewage). **b** Algal distributions in the facultative pond (*middle*). **c** Algal distributions in the outlets of facultative lagoon. **d** Algal distributions in the outflows of the maturation

the facultative pond (>500 kg BOD5/ha) at Vidyaranyapuram during the entire study period, while as many as 21 species were present in the maturation ponds with surface loading of >100 kg BOD5/ha. The relative abundance of the algal species is provided in Table 4.

The key indicators of the unit operation processes are associated with the transition of algal biomass. The distribution of various algal communities in the various unit processes of the pond-based system is provided in Figs. 8a–d and 9. A transition in the species composition towards flagellate forms indicates more facultative conditions and shows a change in effluent quality. The purple pellet of the water samples after centrifugation showed the presence of purple sulfur bacteria indicating the pond transiting to anoxia and reduced performance. The present study reveals that the algal count in the ponds can be directly correlated with the BOD surface loading. Algal count of ~10^6 cells/ml dominated by euglenoid flagellate members in the facultative ponds indicates a loaded facultative pond with variable stability. In contrast, the algal counts at the maturation ponds were of the order of 10^4–10^5 cells/ml but consisted of numerous nonflagellate algae essentially comprising of the chlorophycean members. Therefore, for climatic conditions similar to Mysore (Tropics), maximum algal biomass production is expected to be around 10 kg BOD5/ha. However, the effect of shock loads on the viability and growth of the algal cells is yet to be understood.

Diurnal variation of chemical parameters

The diurnal analysis showed a consistent difference between the inflow and outflow parameters (Table 5). During this period the flow rate varied from 30 to 33 MLD that is roughly around 47 % of the installed plant capacity. At this loading rate, the wastewater flow gets a higher retention period in the STP, which helps in better treatability. The initial part of the lagoon is characterized by constant sludge dislodging from the bottom by the microbial activities. The pH around this region was consistently towards the acidic side (~6) and the ORP was −200 mV.

The periodic DO analysis showed that DO in the subsurface water is negligible and the surface water have, at times, very less surficial DO that would not affect the bottom anaerobic processes (Mahapatra et al. 2011a). DO, owing to higher photosynthesis, progressively increased from morning to midday and then dropped to a minimum (Table 5). This is in agreement with other studies (Kayombo et al. 2000; Tadesse et al. 2004; Amengual-Morro et al. 2012). In HRAP, the DO levels were almost saturated, i.e., from 86 to 98 % (Craggs et al. 2012a).The algal densities at the inlet region were negligible compared to the latter portions of the facultative lagoon. The latter aerobic parts of the facultative lagoon were characterized by higher DO values, 16.25 mg/l (near the outlets of the facultative lagoon), with a consistent high pH value above 8 due to algal photosynthesis. The ORP of these regions were found to be positive (8–59 mV), showing oxidizing conditions at the surface, except the evening sample that showed negative values. A good correlation was observed between the ORP and the pH value (R^2=0.89; p<0.05). These variations could be due to algal, bacterial, and physicochemical stratification in the facultative lagoon's water column, i.e., algal dynamics at different depths. It was observed that the zone of movement of the algal band was strictly restricted to the top 40–50 cm of the pond.

Significant negative correlations were found between the biomass of chlorophyceae with total SS (TSS) and COD. The euglenophyceae members were positively correlated with NH_4-N, total hardness, sodium, and chloride, while diatoms have a negative correlation with the same given parameters. The cyanophyceaen members were negatively

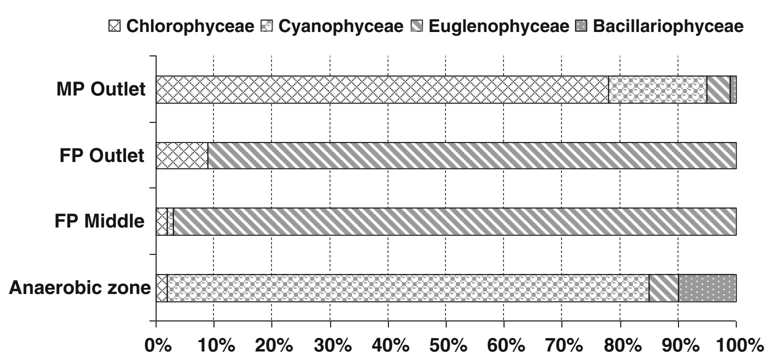

Fig. 9 Algal densities and distribution at different unit processes of STP

Table 5 Diurnal variations of treatment parameters

Sampling sites	Time (am)	pH	WT[a] (°C)	Salinity (ppm)	TDS (ppm)	Elec. cond. (µS)	ORP (mV)	DO (surf) (ppm)	DO (sub surf) (ppm)
Morning-I									
Inflow	08:10	7.25	26	420	680	855	33	2.8	–
FP-01	08:20	6.48	24.3	583	938	1,166	−225	1.09	1
FP-outlet	08:35	7.34	24.4	564	908	1,135	8	2.34	0.34
MP-outlet	08:50	7.66	24.4	567	916	1,147	18	0.53	0.3
Morning-II									
Inflow	11:05	7.18	28.6	505	812	1,018	24	0.18	–
FP-01	10:40	6.45	25.6	554	888	1,110	−212	0.9	0.9
FP-outlet	10:55	8.1	26.1	548	879	1,097	59	12.6	3.6
MP-outlet	10:35	7.66	24.4	567	916	1,147	18	0.53	0.3
Mid day									
Inflow	13:10	7.01	28.9	609	978	1,225	−65	0.27	–
FP-01	12:40	6.34	26.5	567	910	1,140	−221	6.9	1.2
FP-outlet	12:55	8.6	30.1	564	905	1,134	84	16.2	5.2
MP-outlet	12:25	7.41	25.6	586	940	1,178	68	2.73	0.8
Evening									
Inflow	16:40	7.34	28.4	578	932	1,174	−91	0.7	–
FP-01	16:00	6.61	26.4	560	898	1,125	−210	0.59	0.59
FP-outlet	16:20	8.13	29.7	543	871	1,089	48	7.25	3.4
MP-outlet	16:35	7.65	26.3	554	893	1,119	41	0.38	0.12

[a] Water temperature

correlated with pH, water temperature, DO, NH_4-N, and TP (Table 6).

CCA showed four distinct places in the ordinate space characterized by different groups of algal species being impacted by specific physicochemical conditions. Axis 1 accounted for 56.56 % and axis 2 explained 42.57 % of the total variation, respectively, which jointly represents 99.13 %. Correlations between species and environmental parameters were 0.98 and 0.93 for axes 1 and 2, respectively. In Fig. 10, site S1 is dominated by the cyanophycean species followed by bacillariophycean species that are mostly impacted by NH_4-N, Filt.BOD and Filt.COD and TKN values. Site 2 has mainly clustered around nitrate, TSS, and COD. However, sites 3, 4, 5, and 6 are grouped together showing the predominance of euglenophyceae and are affected by the concentrations of DO, pH, alkalinity, and nitrite. On the other hand, S7 were dominated by chlorophycean members.

Bray–Curtis CA aided in the spatial similarity grouping of the seven sites with different bioprocesses and treatment status in the sewage treatment ponds. The four separate clusters illustrate the nutrient concentration with the algal biomass and algal abundance (Fig. 11). Cluster I (94.75 % similarity) comprised of two subclusters, i.e., sites S5 and S6, which indicated 97.5 % similarity and the other subcluster comprising of sites S2 and S3 showed 96.3 % similarity, which were attributed to similarities in the concentrations of DO, pH, and NH_4-N. Sites S4 and S7 had varied characteristics due to differences in their algal assemblage and nutrient concentrations. Site S1 stands out as a separate entity being highly anaerobic with a reduced state of elevated organic matter, nutrient concentrations, and very meager algal concentration. These techniques would eventually help in devising a proficient gradient-specific way for a sustainable wastewater management through efficient monitoring and assessment.

Table 6 Spearman's rank correlation between physicochemical variables and groups of microalgae from sewage treatment ponds

Parameters	Chlorophyceae	Euglenophyceae	Cyanophyceae	Bacillariophyceae
pH	0.56373	0.64286	**−0.95499****	−0.65738
Temperature	0.72739	0.42857	**−0.84688***	−0.35857
Salinity	−0.16366	0.071429	0.054056	−0.17928
TDS	−0.18185	0.32143	−0.090094	−0.29881
TSS	**−0.81832***	0.10714	0.48651	0.17928
EC	−0.054554	−0.10714	0.12613	−0.059761
DO	0.56373	0.64286	**−0.95499****	−0.65738
COD	**−0.87287***	0.14286	0.48651	0.17928
Filt. COD	−0.072739	0.071429	0.18019	0.53785
BOD	−0.76376	0.14286	0.39641	0.059761
Filt. BOD	−0.72739	−0.17857	0.63066	0.17928
Nitrate	−0.90924	−0.071429	0.59462	0.11952
Nitrite	−0.32733	0.42857	−0.16217	0.059761
TKN	−0.72739	−0.071429	0.61264	0.29881
Amm-N	−0.27277	**0.85714***	**−0.88292***	0.65738
OP	−0.20003	0.64286	−0.34236	−0.17928
TP	0.65465	0.46429	**−0.88292***	−0.41833
Alkalinity	−0.46686	0.76719	−0.44371	−0.87671
Carbonates	0.62199	−0.019703	−0.40756	−0.26375
Bicarbonates	−0.80556	0.38188	0.19267	−0.27386
Ca	0.19444	0.10911	−0.41286	−0.63901
Mg	−0.073398	0.5766	−0.47273	−0.72363
Hardness	−0.0091747	**0.81084***	−0.74545	**−0.90453***
Na	0.018185	**0.78571***	−0.73877	**−0.89642***
K	0.26607	0.48651	−0.57273	−0.66332
Chlorides	0.10185	**0.78195***	−0.77067	**−0.85201***
ORP	0.29096	0.64286	−0.66669	−0.29881

Bold figures indicate statistically significant correlations (* $p<0.05$; ** $p<0.01$)

Characteristics of Euglena cells

Several criteria have been developed for evaluating the characteristic properties of the *Euglena* cells found in the culture at equilibrium conditions.

i. **Cell count, (C).** The number or population of cells per ml. of the sample collected was observed to be 8×10^5 cells/ml.

ii. **Cell shape, (S)**. This is the average ratio of length to width of individual cells. The observations showed broadly two different types of cell shapes a) round with diameter of 40–50 μ and b) motile ones with length 90–100 μ and breadth 15–20 μ.

iii. **Cell volume, (Vm).** This is the real volume of a million cells, in mm^3 and is determined by direct microscopic measurements of the cell. There were two forms the palmellar (round forms) and motile long forms as observed through microscopy. For the palmellar forms the cell volume was calculated to be 47569.5 μm^3. The volume of the motile long forms were 28260 μm^3

iv. **Packed Volume, (V_p)**. This is the centrifuged volume in ml of the cells contained in 1 lt of the sample collected after centrifugation at 5000 rpm. The result is a function of cell count, the cell volume and cell shape. The packed volume was recorded to be 3 ml from 1lt. of the sample.

v. **Cell weight, (Wt.)**. This is the dry wt. of the cells in μg per million cells. The cell wt. was calculated to be 0.5 mg/ million cells.

vi. **Cell density, (ρ).** The cell density is derived from the dry wt. of the cells in mg/m^3 of real volume. The cell density was calculated to be 480 g/m^3.

vii. **Cell Index, (I).** The cell index is the packed volume per million cells (Vc/C) and is represented numerically as the packed volume in m^3 of a million cells that is equal to 3.75×10^{-3} ml.

viii. **Cell colour**. Varies from very dark green to pea green to yellow-brown depending upon the cell types and availability of nutrients. Most of the cells were dark green in color.

ix. **Cell types**. Depending on the age and behavior, various types/forms of Euglena can be observed. **a)** Motile forms: the forms which were observed during their movements **b)** Palmellar forms: These are the matured forms which are found in the resting stage. These type of cellular structures encompasses many encystations inside mucilaginous layer **c)** Transitional forms which are the intermediate between motile and mitotic/palmellar stage **d)** Hyaline forms which are the cells without visible chlorophyll **e)** Swarmers the cells which are the motile forms hatching from palmellar forms and finally **f)** Mitotic forms which are the forms undergoing binary fission. The most dense Euglena cell populations are observed to occur when the retention period was around 7 days.

Algal biovolume (V_b) According to the above-mentioned measurements, the biovolume was calculated as equivalents of the number of cells in unit volume expressed as V/V. From the earlier calculations (box provided above), the volume of the round palmellar forms and the long elongated motile cells were 47,569.53 μm^3 and 28,260 μm^3, respectively. Approximately 70 % of the cells were in the palmellar stage and the rest were in the motile phase. The cell number counts were 5.6×10^5 to 2.4×10^6, respectively. Thus, the contribution of the palmellar stage and the elongated motile cells towards biovolume were $2.66\times10^{13}\,\mu m^3$ and $6.78\times10^{12}\,\mu m^3$, respectively, and the total biovolume per liter of the sample collected was calculated as $3.338\times10^{13}\,\mu m^3$.

Algal biomass calculation Ten liters of the water samples was collected and this was centrifuged to get 9.9462 g of wet weight concentrate. The average dry weight was found to be ~0.4 g/l. An estimate for 1 m^3 of the wastewater would contain of around 400 g of the algal biomass. From the conditions pertaining in the facultative pond, it was found that almost 10 % of the pond was in anaerobic conditions and the rest was having phenomenal euglenoide growth. Out of 50,000 m^2 of the surface area of the facultative lagoon, nearly ~5,000 m^2 was in anaerobic conditions. Hence, the remaining 45,000 m^2 had been able to perform uninterrupted photosynthesis yielding very high cell density of the order of ~10^6 cells/ml. It was also noticed that the cells were very dense in the superficial layers of the pond (40–50 cm), where presumably active photosynthesis takes place. Hence, the volume for the peak algal growth (exponential stage) during photosynthesis was found out to be 9,000 m^3. Considering the uniformity in the greenness of the entire

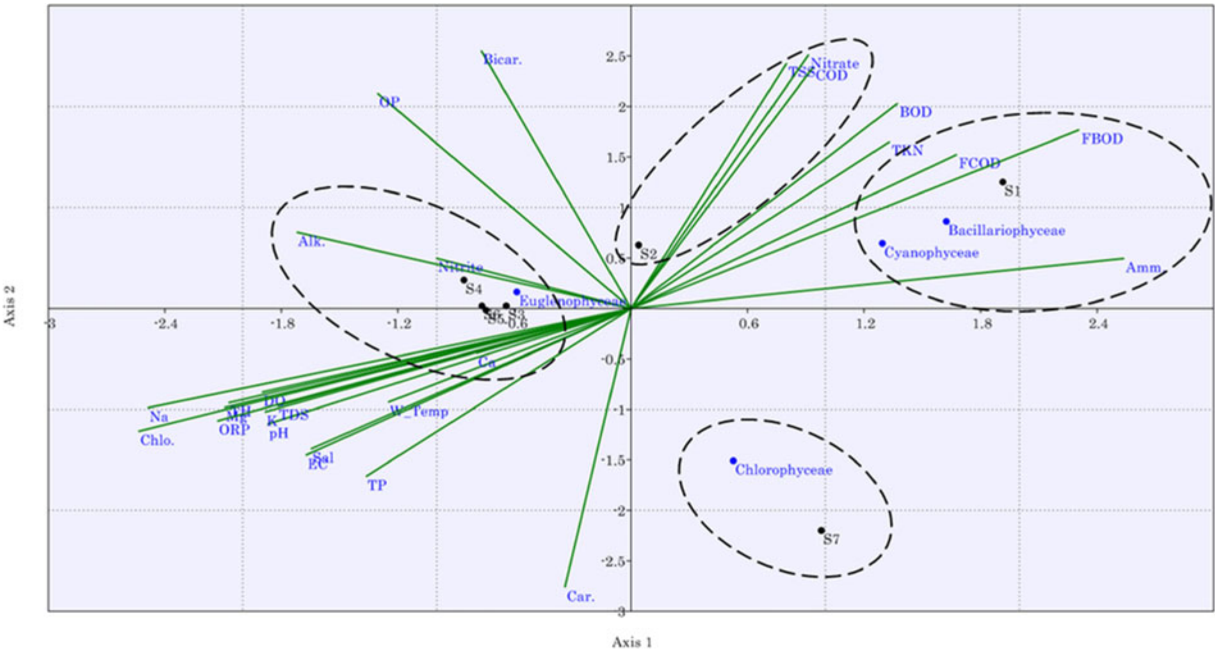

Fig. 10 CCA ordination obtained from algal abundance data for each species and physicochemical variables for the residence time of 12 days in sewage treatment ponds. The ordination of: sampling locations (represented by *points with black dots* from S1 to S7) and physicochemical variables (represented by *green lines*), and micro-algal genera indicated by *blue dots*

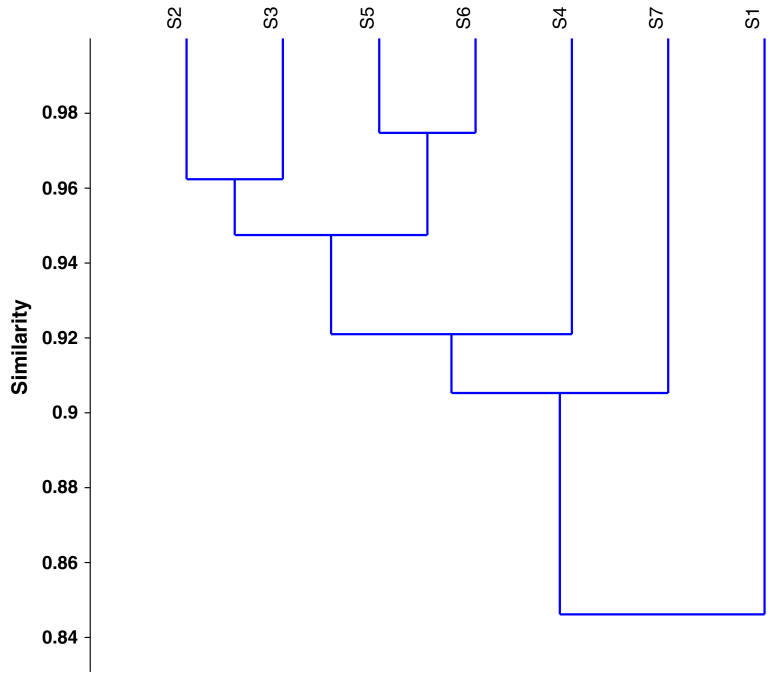

Fig. 11 The similarity in sites through clustering based on physicochemical parameters, algal abundance, and algal distribution in the pond

aerobic region of the pond, the growth was assumed to be uniform throughout. Therefore, 3.5 t of algal matter could be derived in the ponds per week if it is assumed that the average time to reach the exponential phase is 7 days. During the period of peak photosynthesis, 420 t of algal dry weight can be derived. Based on the earlier analysis, where the C content in the case of microalgae was roughly around 40 % (Mahapatra et al. 2011a,b,c), the present concentration of algae yields ~250 g COD/m^3 that is higher compared to ~100 g COD/m^3 (Weatherell et al. 2003) and ~70 g COD/m^3 (Sah et al. 2011).

The C content of the algal biomass was ~168 t of C (average C content in algal biomass was about 40 %) of the standing population of algae. However, this huge quantity of C stock could not have been generated only through photosynthesis but was mostly contributed by heterotrophic mechanism. Therefore, these algae are dependent on the organic C more than the inorganic C leading to a dual mixotrophic life. Here, algae take up the inorganic C during the availability of sunlight autotrophically and in the absence of light, it switches over to a heterotrophic mode. During herterotrophic periods, the algal cells are noticed to store more lipids compared to the phototrophic phases (Xiong et al. 2010) that apportions the total C present into variable carbohydrate and lipids inside the cell depending upon many environmental factors. Studies have shown simultaneous wastewater treatment and algal biofuel production on a hectare scale (Craggs et al. 2012a). However, further analysis is required to understand the heterotrophy of algae and dependence of algae on various biophysical and chemical factors that allocate and sequester organic carbon. Larger population of *Euglena* might even feed on green algae and organic matter rapidly. This might be the reason for lower algal populations of green algae in the facultative ponds. It has been reported that NH_4 stimulates heterotrophic CO_2 fixation in *Euglena*. It has been possible to show that CO_2 fixed heterotrophically by *Euglena* causes the formation of carbon skeletons for amino acid synthesis through tricarboxylic acid cycle and by another less well-defined method (Peak et al. 1980).

Determination of nutrient-integrated efficiencies of the sewage treatment plant

The treatment parameters at different phases of treatment determine the biological decomposition, uptake of nutrients, and effect of the microenvironment on their growth and development. The major factors that affect plant performance are the influent wastewater concentration, the loading rate, the type of microbiota, and the hydraulic detention time. Therefore, devising cumulative efficiency indices would help in the determination of the overall efficiency and hence, it would help in better decision making for further downstream treatment of wastewater.

Several indicators such as TSS, COD, BOD5, and ammonia removal efficiencies are used to comparatively analyze a variety of wastewater treatment systems in sewage firms (Colmenarejo et al. 2006). Nutrient-integrated efficiency is computed considering an average of turbidity, filterable COD, filterable BOD, TN, and TP removal efficiencies. This helps in the evaluation of the extent of treatment from the primary, secondary, and tertiary treatments points of view. This reveals an average nutrient integrated efficiency (NIE) of the effluent, indicating an immediate requirement of an additional detention unit with the oxidative systems that would help the improvement of the NIE values for the wastewaters so that they can be directly discharged into the surface waters or can be directly used for irrigation with a minimal algal count. Oxidative pond-based methods are good in treatment of sewage and removal of microorganisms (Jimenez 2007). Ideally, the efficiency of such systems according to the tropical climate should be closer to 90 %. The newly devised NIE is given by:

$$\begin{aligned} \mathrm{NIE} &= 1/5[\mathrm{E_{TUR}} + \mathrm{E_{COD}} + \mathrm{E_{BOD5}} + \mathrm{E_N} + \mathrm{E_P}] \\ &= 62.8\% \end{aligned}$$

where NIE is in percent,

E_{TUR} is average efficiency of turbidity removal (percent),

E_{COD} is average efficiency of COD removal (percent),

E_{BOD5} is average efficiency of BOD5 removal (percent),

E_{N} is average efficiency of nitrogen removal (percent), and

E_{P} is average efficiency of phosphorus removal (percent).

Conclusions

The present investigations on the treatment efficiencies of the STP showed moderate treatment levels with 60 % total COD removal, 50 % of filterable COD removal, 82 % of total BOD removal, and 70 % of filterable BOD removal. The nitrogen removal efficiency was lower. However, a rapid decrease in the SS after a higher euglenoid growth indicates particulate carbon removal by algal ingestion. Euglenoides dominated the facultative pond and chlorophycean members were more abundant in the maturation ponds owing to variable surface BOD loadings. The transition in algal communities and biomass over the residence time was linked with a shift from anaerobic to aerobic phases, with better organic matter removal. pH and DO were consistently correlated with higher algal abundance. Significant correlations between algal biomass and nutrients indicate the importance of the type and nature of algal communities that can be used as an efficient tool for predicting the dynamics of various phases in wastewater treatment systems. The biovolume calculations for the standing population of the alga in the surface (20 cm) revealed 3.5 tons of algal dry weight accounting to 1.4 t of immobilized carbon in algae, assuming the doubling time of euglenoides was 1 week. The NIE showed a higher shortfall in treatment levels that might be in compliance to a provision of detention of a few days through an additional stabilization pond and an optimal algal management in various phases.

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