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Potential of Using High Rate Algal Pond for Algal Biofuel Production and Wastewater Treatment

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The aim of this study is to investigate the application of high rate algal pond for municipal wastewater treatment and the production of microalgae biomass for biofuel production. Primary facultative pond one cubic meter (1 m^3) followed by high rate algal pond (1.5 m^3) were installed in wastewater treatment plant and fed with municipal wastewater. High rate algal pond enhance the removal of nutrients, organic contaminants, coliforms and *E. coli*. The performance data of the treatment system showed that the removal efficiency of COD, BOD, total suspended solids and ammonia were 31, 33, 22 and 56 %, respectively. The removal of total coliforms reached up to 3 logs. Furthermore, fecal coliforms and *E. coli* removal reached 3 and 5 logs, respectively. The community structure of the high rate algal pond revealed that the most dominant algal species were *Scenedesmes obliquus* and *Micracitinium pusillum*. The total lipid percentage was an average of 5 % in the high rate algal pond. The gas chromatographic analysis of the fatty acid methyl ester revealed that stearic acid (C18:0) concentration reached up to 18.8 %, the oleic acid and linolenic acid were detected with an average percentage of 10.3 % and 13.8 %, respectively. It can be concluded that, the high rate algal pond considered as an efficient and cost-effective pond system for municipal wastewater treatment. Also, it able to produced oil used as biodiesel source from the biomass of the harvested microalgae.

Keywords: High rate algal pond, Wastewater treatment, Algal biofuel, Total coliforms, *E. coli*.

INTRODUCTION

With the world population growth and the phenomenon of global warming rising; water and energy is becoming the most challenging problems facing all world. The fossil oil still is the main source of energy, it account about 88 % of primary energy production [1] but it has been fast diminishing resource due to the huge exploitation by mankind [2]. Many studies explain that, this energy source is expected to be extinct in 50 years [3,4]. Microalgae can be used as an alternative solution for this problem. It has many significant uses *e.g.*, wastewater treatment, organic fertilizer production, clean energy production (*e.g.*, biofuel), greenhouse gas abatement and animal feeds. Therefore, wastewater treatment combined with renewable resource production is the most economically advantageous use for microalgae [5]. Microalgae can produce higher biomass productivity than that produced by plant crops in terms of land area required for cultivation, are predicted to have lower cost per yield and have the potential to reduce green house gases emissions through the replacement of fossil fuels [1,6-10]. However, the capital cost and operational costs for algal biofuel production are presently too expensive [11,12]. One of the main challenges in making algal biomass production economically feasible for its several purposes is reducing input costs (water, nutrient. *etc*.) [13]. Microalgal biomass can be grown as a by-product of high rate algal pond (HRAP) operated for wastewater treatment. Mass cultivation of algae has been studied for over two decades [14] and the most common commercial approach to produce algae biomass on a large scale is an open pond or high rate algae pond system [15,16]. High rate algae pond provides cost effective and efficient wastewater treatment with minimal energy consumption and have a considerable potential upgrade oxidation pond [17,18]. Coupling action by using wastewater treatment with algae cultivation may offer an economically viable and environmental friendly way for sustainable renewable algae-based biofuel and bio-based chemicals production, since large quantities of freshwater and nutrients required for algae growth could be saved and the associated life cycle burdens could be reduced significantly [19]. Primary or secondary treated municipal wastewater is fed to HRAP and mixed with the algae and bacteria. Organic matter removal in a HRAP is achieved by a mutually beneficial between bacteria and algae [20]. The algal photosynthesis produces the oxygen required for the degradation of organic matter by heterotrophic bacteria; whereas the carbon, nitrogen and phosphorus needed for algal growth are provided by bacterial decomposition of wastewater components. A uniform mixing is also required to obtain consistent yield of biomass by ensuring frequent exposure of algae cells to light, avoiding the settling of algae cells to the pond's bottom, homogenizing the nutrient distribution and enhancing the utilization of CO_2 in the pond [21]. Also, the mixing in the HRAP promotes the growth of algae that form colonies which can be easily harvested by gravity settling, additionally, mixing promotes better nutrient distribution [22,23]. To reach the highest degrees of treatment process in HRAP a high degree of separation of algae and bacteria biomass from final effluent [24]. Separation techniques include screening, centrifugation, microstrainers and flotation, although sedimentation without chemical addition is the option commonly adopted in full scale facilities [23]. Thus, the ultimate goal of this study investigates the HRAP efficiency as a municipal wastewater treatment system that utilizes micro-algal growth to simultaneously create renewable energy in the form of biodiesel as well as removing polluting nutrients that facilitate the reuse of the treated wastewater for irrigation.

EXPERIMENTAL

Design and construction of the high rate algal pond (HRAP): This study was conducted using raceway HRAP treating municipal wastewater at El-Zenin treatment plant, Giza governorate, Egypt. The HRAP has surface area of 4.5 m^2, depth of 0.3 m and a total volume of 1.5 m^3 and it is provided by paddle wheel with speed 6 rpm to give a flow rate velocity 0.2 m/s. The HRAP construction and the water flow directions in the pond are shown in the schematic diagram (Fig. 1a & 1b). The hydraulic retention time of the HRAP was 6 days. The HRAP received municipal wastewater from primary facultative pond with 1.5 m deep and hydraulic retention time of 10 days (Fig. 1a). During the first period of the study the research team started to make a full characterization of the domestic wastewater from same location of the study. This sampling program provides a clear picture about the contaminant concentration profile. The characterizations include the physico-chemical, biological and microbiological characteristics.

Biological characteristics

Taxonomic identification: Algal community in wastewater samples were identified up to the species level according to the key of freshwater algae [25,26].

Chlorophyll "a" measurement: The fresh sample of each strain with 25 mL defined volume was taken every 48 h and filtered through 0.45 μm membrane filter and extracted with hot methanol [27] after the addition of 0.5 mL magnesium carbonate solution (1 %) in order to prevent chlorophyll degradation.

The following equation was used for calculating the concentration of chlorophyll a (as μg/L) [28].

$$C_a = 11.85(OD664) - 1.54(OD647) - 0.08\ (OD630)$$

$$\text{Chlorophyll a } (\mu g/L) = \frac{C_a \times \text{Extract volume (L)}}{\text{Volume of sample (L)}}$$

where: OD 664, 647 and 630 are the absorbance at 664, 647 and 630.

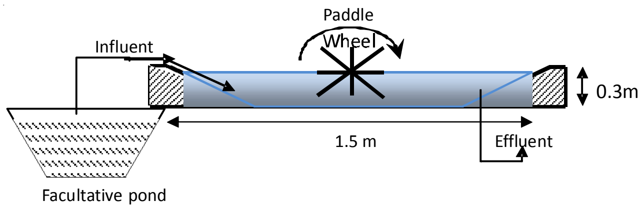

Fig. 1a. Schematic diagram of high rate algal pond

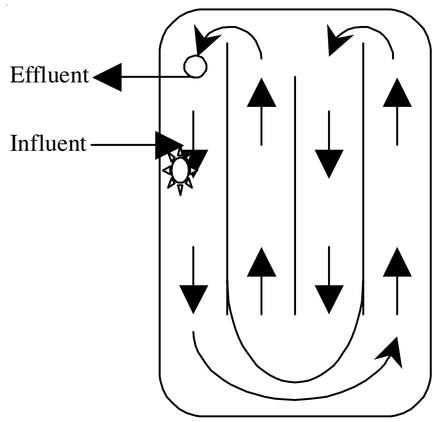

Fig. 1b. Water flow direction in the high rate algal pond

Determination of total lipid content: The oil extraction was carried out according to Bligh and Dyer modified method [29] from the effluent samples. The biomass of microalgae was dried at 60 °C and grind into homogenous fine powder. The dried cells were mixed with methanol-chloroform (1:1, v/v) as a co-solvent using homogenizer for minutes at 800 rpm in a proportion of 1 g in 30 mL of solvent mixture. The homogenate mixture was subjected to a magnetic stirrer at 30 °C for 2 h. The cell residue was removed by filtering. The filtrate was transferred into a separating funnel and sufficient water was added to induce biphasic layering. After settling the solvent mixture was partitioned into two distinct phases, top light green aqueous methanol layer containing most of the co-extracted non-lipids and bottom dark green chloroform layer containing most of the extracted lipids. The chloroform layer was collected in a pre-weighted flask and evaporated using a rotary evaporator.

Bacteriological examination: Total coliforms (TC), fecal coliforms (FC) and *E. coli* (EC) were determined by using MPN method [28]. Total coliforms were detected on lauryl tryptose broth. The inoculated tubes were incubated at 37 °C for 48 h after which acid and gas production was observed and the results considered as a positive presumptive test. Tubes containing BGB were sub-cultured from the positive presumptive tubes and incubated at 37 °C for 48 h. The production of acid and gas were recorded as a positive confirmative test for total coliforms. A few drops from positive tubes of lauryl tryptose broth were inoculated in *E. coli* broth and incubated in circulating water bath at 44.5 °C for 24 h. The production of acid and gas indicate the presence of lactose fermenting fecal coliforms. While, *E. coli* was detected by add few drops of kovacs reagent on positive *E. coli* broth tubes. The results were calculated according to three tubes of MPN table and expressed as MPN-index/100 mL.

Physico-chemical characteristics of wastewater: The performance of the integrated system was monitored twice a week for more than 6 months. The raw wastewater (influent), the facultative pond effluents (effluent 1) and the effluent of HRAP (effluent 2) were analyzed. The pH and temperature were measured regularly *in situ*. The physico-chemical analysis was carried out according to APHA [28] and determine total chemical oxygen demand (COD_{tot}), soluble chemical oxygen demand (COD_{sol}), biochemical oxygen demand (BOD_{tot}), soluble biological oxygen demand (BOD_{sol}), total Kjeldahl nitrogen (TKN), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), total suspended solids (TSS), oil and grease and total phosphorus (TP).

RESULTS AND DISCUSSION

Physico-chemical and microbiological characteristics of raw wastewater: The results obtained from the characterization of the influent municipal wastewater are given in Table-1. From the results obtained, it can be seen that a wide variation in the sewage characteristics are exhibited in terms of chemical oxygen demand (COD), biological oxygen demand (BOD) and total suspended solids (TSS) contents. Municipal wastewater characteristics are not constant in practice but vary. The fluctuation may be seasonally, monthly, daily or hourly. Strength of the municipal wastewater can be classified as medium strength [30]. The average concentration of the COD was 373 mg O_2/L. Corresponding BOD average value was 215 mg O_2/L. BOD_5/COD ratio was 0.58 for raw wastewater. Thus, the biodegradability of the wastewater is more than 50 %. This ratio was given as 0.6 for raw domestic wastewater [31]. The TKN/COD ratio according to the average values was 0. 10 this is relevant with the data given by Rössle and Pretorius [32]. Suspended solids average value was 181 mg/L. Average values of ammonia, organic nitrogen and phosphate were 21 mg NH_3-N/L, 17 mg N/L and 2 mg P/L, respectively. Evaluate of NH_3-N fraction to organic nitrogen for total, wastewaters is 1:0.8. In this study, TKN/COD ratio according to the average value was 0.1, this is also relevant with the data were given by Rössle and Pretorius [32]. In the present study, the bacteriological analysis of the raw wastewater showed that the average values of total coliforms, fecal coliforms and *E. coli* were 2.8×10^6, 5.0×10^5 and 2.8×10^5 MPN-index/100 mL, respectively (Table-1).

Performance of the high rate algal pond (HRAP)

Physico-chemical characteristics of the treated effluent: Wastewater treatment using HRAP was part of an advanced Pond system which comprised primary facultative pond which incorporated anaerobic digestion pits and HRAP pond in series. The integrated system operated for more than 6 months (186 days) under the normal Egyptian weather conditions. The hydraulic retention time for the facultative pond was 10 days and for HRAP it was 6 days. The characterization of the effluents of the two ponds concentrations were shown in Table-1. The results showed that the pH increases to average of 9.2 after HRAP that could be explained by limitation of CO_2 during daytime algal photosynthesis [17]. The performance of the treatment system showed that, the total COD_{tot} and COD_{sol} removal percentage were 51 and 54 %, respectively after the facultative pond. The removal percentage was due to the settling of the total suspended solids in the facultative pond and accumulated at the bottom together with settled dead micro-organisms they undergo anaerobic digestion. The removal percentage became negative (-45 %) for COD_{sol} after the HRAP this was due to the algae production. The overall removal percentage of the integrated system (facultative pond + HRAP) was 31 % for total COD_{tot} and 27 % for COD_{sol} with an average residual concentration 237 and 102 mg O_2/L, respectively. BOD_{tot} removal percentage reaches 57 % but decrease to 51 % after HRAP this refers to the increased production of algae in the effluent, but the average total BOD removal percentage was 33 %.

Total suspended solid removal percentage was 54 % after the primary facultative pond but the effluent of the HRAP gave negative removal percentage and the overall removal

TABLE-1
PHYSICO-CHEMICAL AND BACTERIOLOGICAL CHARACTERISTICS

Parameter	Influent		*Effluent 1			**Effluent 2			Total (% R)	Ministerial decree for reuse primary treatment
	Average	± SD	Average	± SD	% R Fac. P	Average	± SD	% R HRAP		
pH	7.5	0.2	7. 9	0.3	–	9.2	0.4	–	–	–
COD_{tot} (mg O_2/L)	373	119	159	66	51	237	87	-49	31	600
COD_{sol} (mg O_2/L)	163	58	76	35	54	102	31	-45	27	–
BOD_{tot} (mg O_2/L)	215	70	86	4	57	130	56	-51	33	300
BOD_{sol} (mg O_2/L)	113	47	42	17	58	52	22	-28	47	–
TSS (mg/L)	181	48	74	37	54	137	105	-119	22	350
TKN (mg/L)	38	11	31	11	19	22	12	32	45	–
Ammonia (mg/L)	21	5	16	5	23	10	6	42	56	–
Nitrite (mg/L)	0.12	0.1	0.18	0.41	–	0.9	1.28	–	–	–
Nitrate (mg/L)	0.11	0.2	0.24	0.4	–	0.6	0.8	–	–	–
Phosphorous (mg/L)	2	± 1	1	± 1	12	2	± 1	38	35	–
Total coliforms (MPN-index/100 mL)	2.8×10^6	4.5×10^6	8.0×10^3	1.0×10^4	99.7	3.7×10^3	4.5×10^3	55	99.8	Unlimited
Fecal coliforms (MPN-index/100 mL)	5.0×10^5	6.8×10^5	2.6×10^3	3.8×10^3	99.4	2.6×10^2	3.5×10^2	90	99.9	1000 MPN/100 mL
E. coli (MPN-index/100 mL)	2.8×10^5	4.8×10^5	1.8×10^3	3.0×10^3	99.3	35	44	98	99.9	–

TKN = Total Kjeldahl nitrogen

percentage was only 22 % and residual average concentration 137 mg/L (Table-1). The HRAP removed high percentage of nutrients 42 % of ammonia, 32 % of total Kjeldahl nitrogen and 38 % of phosphorous. Microalage enhanced the removal of significant amount of nutrients (N and P) because they required high amounts for proteins, nucleic acid and phospholipids synthesis. Also; the nutrients removal enhanced by the increase of pH level which is associated with photosynthesis which lead to stripping of ammonia and phosphorous precipitation [33]. From the previous results; we can conclude that the treated effluent complying with the first degree of treatment thus it can be safely used for irrigation wooden trees. Thus, it is essential to have an efficient algal biomass harvesting to achieve a high quality treated effluent that could be safely reused without restrictions.

Bacteriological examination: Bacterial indicator containing total coliforms (TC), fecal coliforms (FC) and *E. coli* are still gold standard for assessment of water and wastewater treatment processes [34,35]. The bacteriological analysis results were given in Table-1. The removal percentage of total coliforms, fecal coliforms and *E. coli* were 99.8 and 99.9 %, respectively. Wastewater treatment containing total coliforms, fecal coliforms and *E. coli* bacteria with active sludge can be effective, with reduction percent reaching up to 99 % or more [36]. The high removal percentage could be explained that the microalgae enhance the deactivation of fecal bacterial indicator by a raising the pH value due to rapid photosynthesis of microalgae that consumes CO_2 faster than producing by bacterial respiration, the temperature and dissolved oxygen concentration of the treated effluent [37-39]. Another explanation for the high removal of coliform bacteria in the effluent may be due to the intensity of the sun light that able to damage fecal bacterial indicator by being absorbed by the humic substances ubiquitous in wastewater [40]. In the current research, the average value of fecal coliforms was 2.6×10^2 MPN-index/100 mL in the final treated effluent (Table-1). The bacteriological results showed that, the treated wastewater was complying with the Egyptian Standard for mixing with the surface water and using for irrigation purposes whereas, the average value of fecal coliforms was 2.6×10^2 MPN-index/100 mL. It well known that, the recommended value of fecal coliforms counts in the treated effluent to be matched with the guidelines for discharging and mixing with the natural surface water is 1000 CFU/100 mL [41,42].

Algal community structure in high rate algal pond: The HRAP is an open pond which is difficult to control the culture conditions, thus only few microalgae species can be successfully dominant. At the beginning of HRAP operation the algal community structure showed that, it contains algal species belonging to algal group of *Chlorophyta, Euglenophyta, Cyanophyta* and *Bacillariophyta*. The most dominant algal species were *Scenedesmus obliquus, Micractinium pusillum, Dictyosphaerium pulchellum* and *Coelastrum sp* (Table-2). After about three month's operation, the pond began to be dominant with *Scenedesmus quadricauda* mainly in addition to *Scenedesmus obliquus* and *Micractinium pusillum*. The other groups of algae were rare or not detected. The variation in community structure may be due to variation in temperature in different season in addition to the ability of certain species to grow fast and be dominant rather than other species.

TABLE-2
COMMUNITY STRUCTURE AND SPECIES DOMINANCY

Algal taxa	High rate algal pond	Effluent
Chlorophyta		
*Ankistrodesmus acicularis (*A. Braun)	+	–
Chlamydomonus variabills (Ehrenb.)	+	+
Chlorella vulgaris (Beyerinck)	+	–
Coelosterum microporum (Skuja)	++	+
Dictosphaerium pulchellum	++	+
Kirchneriella obese	+	±
Oocystis parva	+	–
Scenedesmus obliquus (Hortob)	+++	+
Scenedesmus quadricauda (Hortob)	++	+
Tetrastrum staurogeniiforme (Schröder)	–	+
Micractinium pusillum	+++	+
Euglenophyta		
Euglena variabilis (Ehrenberg)	±	–
Haematococcus pluvialis	++	+
Pandorina	+	–
Cyanophyta		
Merismopedia elegans (Braun)	+	+
Oscillatoria agardhii (Gomont)	++	+
Oscillatoria limnetica (Limmermann)	++	+
Microcystis aeruginosa	+	±
Phormedium rimosum	+	–
Bacillariophyta		
Cyclotella comta (Kutz.)	±	–
Gomphonema oilvecum	++	±
Navicula gastrum	+	–
Nitzschia linearis	+	–
Stephanodiscus dubius (Fricke)	+	±

++++: Dominant; +++: Plenty; ++: Many; +: Appreciable; ±: Rare; –: not detected.

Growth measurement: Chlorophyll a was measured in order to evaluate the growth rate of the community structure in the HRAP since this parameter is widely recognized to be directly correlated with algal biomass density [43]. The chlorophyll readings varied from one day to another and the maximum reading reached 16430.6 µg/L in the pond after 52 days (Fig. 2), this may be due to increasing temperature at this time since this reading was at August, since algal productivity increases with increasing pond temperature which increasing algal respiration and photorespiration reduce overall productivity [5,6,44,45]. The average of the readings was 1543.1 µg/L. Also, the chlorophyll of the effluent was detected and reached the maximum reading in 72nd days with maximum reading 4048 µg/L.

Total lipid percentage: The total lipid percentage of the microalgae collected from the pond was ranged from 1.9 % ± 0.0 at the first week and reached to 6 % ± 0.3 after about 23 weeks. Although the results of Abdo *et al.* [46] showed that *Scenedesmus quadricauda* has a total lipid percentage 16 %, the maximum reading was 9 % ± 0.5 when the HARP was dominant with *Scenedesmus quadricauda* as shown in Fig. 3. The average of all reading during 23 weeks was 5 % ± 2.4. Since nitrogen is a critical factor for regulating algal cell lipid content [45,47,48], this variation in lipid content may be due to nitrogen concentration on HRAP. The production of biofuel from microalgal biomass needs two important factors first high lipid content and second high biomass production. In this study

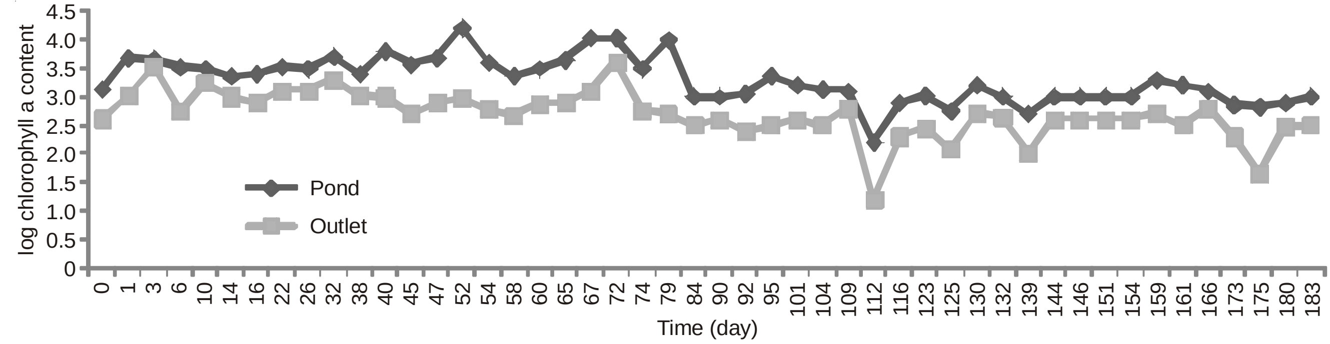

Fig. 2. Growth curve of high rate algal pond

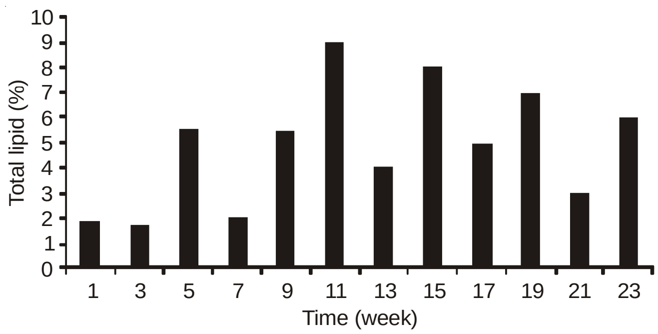

Fig. 3. Total lipid (%) of high rate algal pond

biomass production was 1 g/L/day. So many works could be done in order to increase biomass and lipid content of the HRAP.

GC analysis of oil samples: Table-3 showed the fatty acid methyl ester (FAME) of oil sample collected from HARP when total lipid was ranged between 5 and 9 %. When the lipid content was 5 % the community structure of algae was mixed and dominant mainly with *Scenedesmus obliquus, Micractinium pusillum* and *Scenedesmus quadricauda.* The results showed that palmitic acid percentage (C16:0) reached 34 % followed by stearic acid 18.8 %. Referring to unsaturated fatty acids both oleic and linolenic acid were found in high percentage 10.3 % and 13.8 % respectively, in addition linolenic acid was found but in small percentage 1.9 %.

When the community structure was dominant with *Scenedesmus quadricauda* mainly the total lipid percentage reached 9 %. As shown in Table-3, the GC analysis showed that palmitic acid percentage reached 47.1 %, which was higher than that detected when lipid percentage was 5 %. The identified fractions of both samples could be used as a source of biodiesel since the target fractions (especially C16:0) were found in high percentages and the percentage of saturated fatty acids was higher than unsaturated fatty acids.

TABLE-3
FATTY ACID PROFILE OF SAMPLES COLLECTED FROM HIGH RATE ALGAL POND

Fatty acids	Common name	A	B
C:12:0	Lauric acid	3.1	2.0
C:14:0	Myristic acid	-	4.7
C:14:1	Myristoleic acid	-	1.6
C:16:0	Palmitic acid	34	47.1
C:17:0	Margaric acid	2.9	2
C:18:0	Stearic acid	18.8	5
C:16:1	Palmetolic acid	10.4	4
C:18:1	Oleic acid	10.3	2.8
C:18:2	Linoleic acid	1.9	3.7
C:18:3	Linolenic acid	13.8	5.5
Total fatty acids identified (%)		95.2	78.4
Saturated fatty acids (%)		58.8	60.8
Unsaturated fatty acids (%)		36.4	17.6

A = Total lipid 5 % ± 2.4; B = Total lipid 9 % ± 0.5.

Conclusion

From the results, it can be concluded that the high rate algal pond (HRAP) proved to be an efficient and a cost-effective pond system for municipal wastewater treatment. The HRAP provides improvement of natural disinfection, also it provides nutrients and organic pollutants removal but the system still difficult of harvest the biomass formed in the pond. Increasing harvested biomass and biofuel conversion from the harvested microalgae could provide valuable cost coverage of the construction, high land requirement of open system and biomass harvesting.

REFERENCES

1. L. Brennan and P. Owende, *Renew. Sustain. Energy Rev.*, **14**, 557 (2010).
2. A. Srivastava and R. Prasad, *Renew. Sustain. Energy Rev.*, **4**, 111 (2000).
3. Y. Chisti, *Biotechnol. Adv.*, **25**, 294 (2007).
4. S. Shafiee and E. Topal, *Energy Policy*, **37**, 181 (2009).
5. J.P.K. Park, R.J. Craggs and A. Shilton, *Bioresour. Technol.*, **102**, 35 (2011).
6. J. Sheehan, T. Dunahay, J. Benemann and P. Roessler, National Renewable Energy Laboratory, NREL/TP-580-24190 (1998).
7. Y. Chisti, *Trends Biotechnol.*, **26**, 126 (2008).
8. D.E. Brune, T.J. Lundquist and J.R. Benemann, *J. Environ. Eng.*, **135**, 1136 (2009).
9. E. Stephens, I.L. Ross, Z. King, J.H. Mussgnug, O. Kruse, C. Posten, M.A. Borowitzka and B. Hankamer, *Nat. Biotechnol.*, **28**, 126 (2010).
10. G.K. Pittman, A.P. Dean and O. Osundeko, *Bioresour. Technol.*, **102**, 17 (2011).
11. J.R. Benemann, Singapore, November 17–18, p. 15 (2008).
12. M. Tampier, Suitability and Barriers to Implementation Prepared for the British Columbia Innovation Council. January 14 (2009).
13. R.H. Wijffels, M.J. Barbosa and M.H.M. Eppink, *Bioprod. Biorefining*, **4**, 287 (2010).
14. A. Richmond, A. Vonshak and S.M. Arad, Elsevier/North Holland Biomedical Press, Amsterdam, The Netherlands, pp. 65–73 (1980).
15. J.C. Weissman, R.P. Goebel and J.R. Benemann, *Biotechnol. Bioeng.*, **31**, 336 (1988).
16. A. Richmond, *J. Appl. Phycol.*, **4**, 281 (1992).
17. S. Heubeck, R.J. Craggs and A. Shilton, *Water Sci. Technol.*, **55**, 193 (2007).
18. J.B.K. Park and R.J. Craggs, *Water Sci. Technol.*, **61**, 633 (2010).
19. W. Zhou, P. Chen, M. Min, X. Ma, J. Wang, R. Griffith, F. Hussain, P. Peng, Q. Xie, Y. Li, J. Shi, J. Meng and R. Ruan, *Renew. Sustain. Energy Rev.*, **36**, 256 (2014).

20. A. Abeliovich, Handbook of Microalgal Mass Culture, CRC Press, Boca Raton, FL, pp. 331-338 (1986).
21. D.E. Brune, G. Schwartz, A.G. Eversole, J.A. Collier and T.E. Schwedler, *Aquacult. Eng.*, **28**, 65 (2003).
22. W.J. Oswald, in eds.: C.A. Lembi and J.R. Waaland, The Role of Microalgae in Liquid Waste Treatment and Reclamation Algae and Human Affairs, Cambridge University Press, Cambridge, pp. 255-282 (1988).
23. W.J. Oswald, *Water Sci. Technol.*, **24**, 1 (1991).
24. J. García, R. Mujeriego and M. Hernández, *Ingeniería del Agua*, **5**, 35 (1998).
25. J.T. Komárek, B. Fott and G. Huber-Pestalozzi, Das Phytoplankton des Süsswassers, *Die Binnengewässer* 16, 7/1: 1-1044, Schweizerbart Verlag, Stuttgart, Deutschland (1983).
26. J.T. Komárek and K. Anagnostidis, in eds.: H. Ettl, G. Gärtner, H. Heynigh and D. Mollenhauer, Süsswasserflora von Mitteleuropa, 19/1 Gustav Fischer, Jena-Stuttgart-Lübeck-Ulm, 548 pp. (1998)..
27. S.M. Abdo, M.H. Hetta, F.A. Samhan, R.A.S. El Din and G.H. Ali, *Asian J. Plant Sci*., **11**, 109 (2012).
28. APHA, American Public Health Association, Standard Methods for the Examination of Water and Wastewater, Washington, D.C., edn 22 (2012).
29. E.G. Bligh and W.J.A. Dyer, *Can. J. Biochem. Physiol.*, **37**, 911 (1959).
30. A. Metcalf and A. Eddy, Treatment and Reuse, McGraw-Hill. New, York. USA, edn 4 (2003).
31. R. Íleri, Environmental Biotechnology, Degisim Yayinlari, pp. 160 (2000).
32. W.H. Rössle and W.A. Pretorius, *Water S.A.*, **27**, 405 (2001).
33. K. Chojnacka, A. Chojnacki and H. Górecka, *Chemosphere*, **59**, 75 (2005).
34. G. Bitton, Florida Storm Water Association, pp. 1-71 (2005).
35. M.A. El-Liethy, Ph.D. Thesis, Microbiology Department, Faculty of Science, Ain Shams University, Egypt (2013).
36. Z. Paluszak, A. Ligocka and B. Breza-Boruta, *Pol. J. Environ. Stud.*, **12**, 345 (2003).
37. P.K. Robison, Y.S. Wong and F.Y. Tamnora, Wastewater Treatment with Algae, Springer and Landes Bioscience, pp. 1-16 (1998).
38. G. Schumacher, T. Blumem and I. Sekoulov, *Water Sci. Technol.*, **47**, 195 (2003).
39. S. Elshafai, F. Elgohary, F. Nasr, N. Petervandersteen and H. Gijzen, *Bioresour. Technol.*, **98**, 798 (2007).
40. R.J. Craggs, A. Zwart, J.W. Nagels and R.J. Davies-Colley, *Ecol. Eng.*, **22**, 113 (2004).
41. USEPA, Status Report EPA-823-R-03-008, US Environmental Protection Agency, Washington D.C. (2003).
42. WHO, Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture, Geneva, Report No. 778 (1989).
43. C.S. Reynolds, in eds.: M. Usher, D. Saunders, R. Peet and A. Dobson, Ecology of Phytoplankton, Cambridge University Press, Cambridge, p. 535 (2006).
44. D.M. Tillett, Ph.D. Thesis, The School of Chemical Engineering, Georgia Institute of Technology, USA (1988).
45. O. Pulz, *Appl. Microbiol. Biotechnol.*, **57**, 287 (2001).
46. S.M. Abdo, E. Ahmed, S.A. El-Enin, R.S. El-Din, G. El-Diwani and G.H. Ali, *J. Algal Biomass Utln.*, **4**, 51 (2013).
47. K.E. Cooksey, J.B. Guckert, S.A. Williams and P.R. Callis, *J. Microbiol. Methods*, **6**, 333 (1987).
48. M.J. Griffiths and S.T.L. Harrison, *J. Appl. Phycol*., **21**, 493 (2009).