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Research Article
Enhancing Biomass, Energy and Value Added Compounds Yield from Pilot Scale Pond System

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Abstract

Background and Objective: High rate algal ponds (HRAP) is a promising technology in wastewater treatment and algal biomass production. It is considered as a cost-effective as it covers the capital and operation costs by recovers of water and nutrient which is beneficial in agriculture. Also, algal biomass produced could be used for the production of valuable raw materials. The aim of this study was to evaluate the application feasibility of HRAP in Egypt for economical and low energy wastewater treatment combined with potential production of algal biomass for biofuel and value added compounds. **Materials and Methods:** The integrated system applied was Facultative Pond (FP)+HRAP+Tube Settler (TS). The FP effluent was fed to HRAP which breakdown the soluble organic substances, provides good natural disinfection and produce algal biomass. Finally, HRAP effluent fed to TS which remove about 60% of TSS. **Results:** The results indicated that the integrated system was efficient in removing COD, BOD and TSS, average removal percentages were 61, 66 and 65%, respectively. The HRAP increases the algal biomass growth, where, the average algal biomass was 27g m^{-2}/day. It was cleared that FP and HRAP ponds proved to be an excellent process for bacterial removal; the coliform group removal was ranging between 3 and 4 logs. **Conclusion:** The results proved that the final effluent characterization is complying with Egyptian Ministerial Decree for reuse.

Key words: Wastewater treatment, algal biomass, coliform group, biofuel and pigments, high rate algal pond, tube settler

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Water and energy are considered two of the main pillars of human civilization. Rapid population growth has led to the worsening of the water scarcity problem and increasing fossil fuel uses. As the population increase and the economy expand there will be increased consumption in fossil fuel and as a result increase the atmospheric concentration of CO_2 as well as greenhouse gas[1]. These prove that the world facing water, energy and environmental disaster which need an urgent solution. In developing countries, the untreated municipal wastewater poses the main contamination cause of water natural resources, which gave rise to concerns about appropriate wastewater treatment management[1].

Wastewater treatment and resource recovery using microalgal-bacterial based systems has been the focus of international research over the last couple of decades[2,3]. Coupling biological wastewater treatments with microalgae cultivation is supposed to be a win-win strategy to tackle this challenge due to the advantages of reducing the energy use and emissions during treatment[4], production of low-cost biomass for bioenergy generation[5] and recovery of important nutrients like nitrogen and phosphorus[6].

Algae growing in wastewater treatment consume nutrients and thus sub-sequent harvest of the algal biomass recovers the nutrients from the wastewater[7-9] and in the same time it considered a good source of biodiesel and other valuable biomolecules which produced from the algal biomass. The intensive coupling of microalgae production with municipal wastewater treatment could generate significant quantities of biodiesel in an economic way and will not affect the environment since large amounts of freshwater and nutrient required for algal growth could be saved[10]. Also, municipal wastewater is rich in ammonia (NH_3), phosphate (PO_4) and other essential nutrients that are required to support microalgal biomass production, in addition to trace metals essential for photosynthesis such as Fe, Cu, Mn and Zn[11].

High rate algal pond (HRAP) is an attractive choice that replace conventional treatment systems, due to its advantages. It requires less than half the land of conventional stabilization ponds. It uses solar energy efficiently for oxygenation and hence, requires from 50-75% less electricity than mechanical aeration of sewage. The activity of stabilization pond is a complex process involving algae, protozoa and bacteria to stabilize wastewater and reduce bacterial count[12]. It provides advanced degrees of treatment at little or no addition cost to its low construction, operation and maintenance costs[13]. Algal growth leads to increasing pH and toxic forms of oxygen concentration both of which bactericidal to Fecal coliform[14,15]. The effluent of a high-rate pond (HRP) contain high concentration of algal suspended solids (SS), which must be separated, thus the treated wastewater reaches the specification for reuse in different purposes. The harvesting step of algae is the higher cost step in the process it remains the foremost obstacle to industrial scale processing. Using gravity sedimentation is usually known as the first method in algal wastewater treatment systems. Different processes for harvesting are used which include chemical, mechanical, physical and electrical, however, combination or sequence of these processes are commonly in use[16].

The aim of this work was to study the treatment of domestic wastewater using HRAP and the harvesting of algae from pond effluent using Tube Settler (TS). Also studying the removal of fecal coliform in the treatment pond to reach the Egyptian legislation for treated wastewater reuse. On the other hand, the use of algal biomass for production of valuable raw materials.

MATERIALS AND METHODS

Pilot scale set-up was located at municipal wastewater treatment plant (Zinin/Giza/Egypt).

The integrated system consisted of primary Facultative pond (FP) followed High Rate Algal Pond (HRAP) and Tube Settler (TS) (Fig. 1). The FP volume was 1 m^3 with a detention time 5 day, it was fed with screened municipal wastewater. The HRAP according to Doma *et al.*[17] 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 sec^{-1} (Fig. 2). The detention time was 4 days.

The tube settler was designed and constructed for separation of algal biomass from the HRAP effluent with volume of 0.25 m^3 (Fig. 2). It was successfully operated for almost a year without operational problem. The settler consists of two compartments separated with inlet channel, each compartment consisted of a 30 tubes installed at about 55-60°C to the surface of the tank. The HRAP effluent directed to flow up through the settler to the surface of the tank. After the algae have been removed and collected on the tube they slide down to the sludge zone.

Physicochemical characters: Physico-chemical analyses were carried out according to APHA., AWWA and WEF[18] to determine total chemical oxygen demand (COD_{tot}), biological oxygen demand (BOD_{tot}), total suspended solids (TSS), total Kjeldahl (TKN), ammonia (NH_3-N), total phosphorous (TP). Algal biomass separated by tube settler was estimated as sludge analysis.

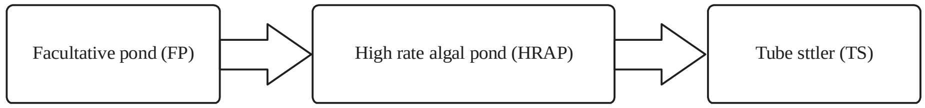

Fig. 1: Schematic diagram of the treatment process

(a)

(b)

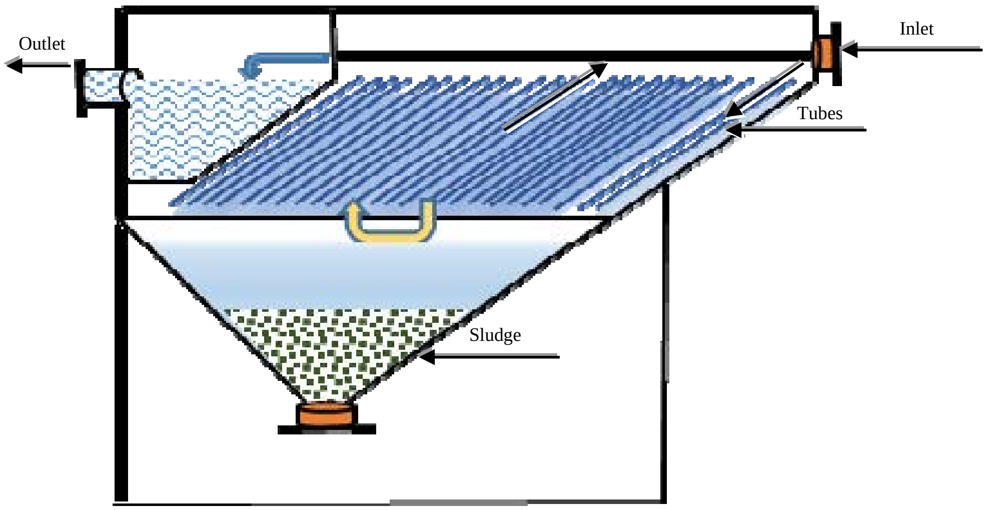

Fig. 2(a-b): (a) High rate algal pond (HRAP) and (b) Tube settler

Bacteriological examination: Bacteriological analysis of coliform group (TC, FC and *E. coli*) was performed by using multiple tube fermentation technique according to APHA., AWWA and WEF[18]. Results were reported as a MPN-index/100 mL.

Biological characteristics

Measurement of algal chlorophyll-a content: The growth rate of algal biomass was assessed by determining the chlorophyll a content according to APHA., AWWA and WEF[18].

Identification of algal community structure: Lugol's-iodine solution was used to preserve the algal sample. Subsamples were dispensed into glass Sedgwick-Rafter cells and examined using OLYMPUS CX41 microscope. Species composition and dominance in the samples were determined semi-quantitatively. Algal identification has been done according to the main references used in phytoplankton identification[19-22].

Extraction of carotenoid: About 10 mL distilled water was added to 10 g of dry algae cells to form a slurry, 5 g of dry granular citric acid was further added to the slurry, then Jojoba oil was added as (1:1) w/w of algal biomass. The mixture was homogenized at 1000 rpm for approximately 15 min, [Homogenizer Model Wise Tis HG-150, Germany]. Centrifugation of the mixture to obtain two layers, the upper layer was the carotenoid with jojoba oil.

Determination of total lipid content

Lipid extraction: Samples after harvesting were subjected to oil extraction as follows:

- **Hexane-Isopropanol extraction method:** The biomass of microalgae was dried and ground into homogenous fine powder. The dry cells were mixed with hexane-Isopropanol (3:2, v/v)[23].
- **Transesterification of oil to biodiesel:** The reaction was conducted according to D'Oca *et al.*[24] using H_2SO_4 acid (98%) as a catalyst (100% in relation to the mass of lipid)
- **Fuel properties after blending with gasoline (10%):** Fuel properties such as density, viscosity, cetane index, flash point and pour point was analyzed and measured after blended with 10% gasoline in the Egyptian Petroleum Research Institute, Cairo, Egypt, according to ASTM[25].

RESULTS AND DISCUSSION

Wastewater characteristics: The physicochemical characteristics of the domestic wastewater recorded in Table 1. The wastewater contains high concentration of organic matter as demonstrated by the chemical oxygen demand (COD) and biological oxygen demand (BOD_5) values. The average COD was 324 and 78 mg O_2 L^{-1} for influent and settler, respectively. The BOD_5/COD average ratio values was 0.37. Thus, the biodegradability of the domestic wastewaters is around 40%. Total suspended solids average value was 168 mg L^{-1} for influent. Average values of ammonia and organic nitrogen were 23 mg NH_3-N/L and 28 mg N L^{-1}, respectively. The ratio of inorganic nitrogen and phosphorous (N/P) was 10.8 this ratio is almost the same recorded by Darley[26] and Martin *et al.*[27], who suggested that the ratio of inorganic N/P for fresh water algae growth was in the range of 6.8-10.

Performance of the integrated treatment system: To evaluate the performance of the integrated system; Facultative Pond (FP), High Rate Algal Pond (HRAP) and Tube Settler (TS), they were operated for one year under ambient weather temperature and it was fed with natural municipal wastewater. The FP used 5 days 'detention time during study period. The FP which combines two different operating processes, aerobic at the surface and anaerobic at the base of the pond with the settlement of sludge[28], indicates that the facultative ponds normally follow anaerobic ponds. The pond was efficient in removing COD, BOD and TSS the average residual concentration was 120, 61 and 54 mg L^{-1} and average removal percentages were 61, 66 and 65%, respectively (Table 1). These results are in contrary with HRAP effluent characterizations which showed increasing in concentration of COD, BOD and TSS than FP effluent with an average 213, 108 and 96 mg L^{-1}, respectively (Table 1), this could be attributed to the excessive production of algal biomass which is accepted as organic matter, could contribute to increasing COD and TSS loads in the HRAP effluent (Fig. 3, 4), similar results have been reported by Pano and Middlebrooks[29] and Jalali *et al.*[30].

Average removal of ammonia and organic nitrogen was 21 and 13%, respectively in FP effluent and about 17 and13%, respectively in HRAP effluent. Ammonia-N removal occur through mainly three process (1) Gaseous ammonia stripping to the atmosphere (2) Ammonia assimilation in algal biomass (3) Biological nitrification. The recorded results in Table 1 showed low concentration of nitrite and nitrate in both ponds effluents indicated that nitrification doesn't represent the main process of ammonia-N stripping. The basic factor in nutrients removal and bacteriological removal is increasing in pH at HRAP pond the pH was $\geq$8.4 and $\geq$7.8 at FP effluent, alkaline pH shifts the equilibrium equation to gaseous ammonia production, whereas, mixing conditions affect the biomass transfer coefficient and temperature affects both of them[31]. As, the nutrients removal mainly depends on two main factors, first direct factor which is algal growth as well as subsequent biomass separation. Second factor is indirect factor; which is resulted from raising the pH of HRAP by algal photosynthesis results stripping of ammonia and precipitation of orthophosphate causing indirect removal of nutrients[7].

Table 1: Average physico-chemical characteristics of integrated pond system

Parameters	Units	Raw (Inf.) average	FP (Eff1) Average	FP (Eff1) R (%)	HRAP (Eff2) Average	HRAP (Eff2) R (%)	TS (Sett.) Average	TS (Sett.) R (%)	Ministerial decree Class B
pH		7.60±1.20	7.80±0.4		8.4±0.6		8.00±0.4		6-9
Chemical oxygen demand (COD)	mg O_2/L	324.00±60.6	120.00±36.4	61	213.0±56.3	-85	78.00±31	55	80
Biological oxygen demand (BOD_5)	mg O_2/L	184.00±31.5	61.00±23.8	66	108.0±38.6	-93	45.00±17	56	60
Total suspended solids (TSS)	mg L^{-1}/LTSS/L	168.00±34.6	54.00±20	65	96.0±36	-80	39.00±16	58	50
Phosphorous	mg L^{-1}	2.20±0.8	1.20±0.4		2.0±1.6		1.60±1		
Ammonia	mg L^{-1}	23.60±4.9	21.00±8.4	21	19.0±9.4	17	6.00±5.5	67	
Organic nitrogen	mg L^{-1}	28.00±10.6	24.00±3.5	13	24.9±5.4	13	9.30±4.8	59	
Nitrite	mg L^{-1}	0.04±0.04	0.14±0.17		0.3±0.43		0.12±0.26		
Nitrate	mg L^{-1}	0.46±0.61	0.80±0.4		0.6±0.3		0.30±0.47		
Fecal coliform	MPN-index/100 mL	2.7E+07	3.05E+04		6.7E+01		1.5E+00		5000

HRAP: High rate algal pond effluent TS: Tube settler effluent

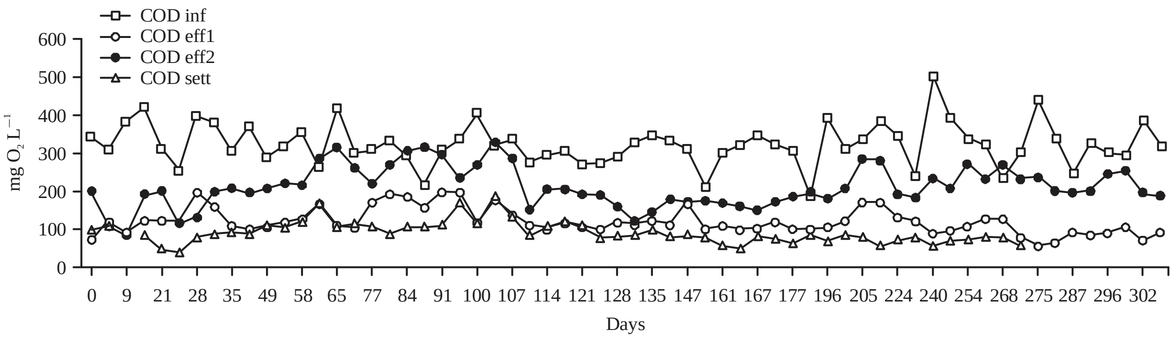

Fig. 3: COD concentrations during the different treatment steps

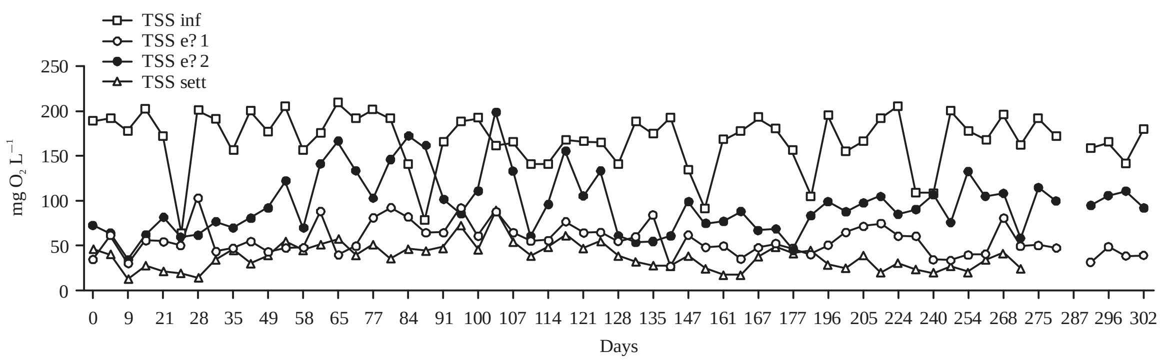

Fig. 4: TSS concentration during the different treatment steps

To improve the treated wastewater quality complies with national legislation of water reuse and also to collect the maximum amount of the microalgae biomass; HRAP effluent was fed to tube settler with 24 h detention time. The results recorded in Table 1, proved that the final effluent characterization is complying with ministerial decree for reuse and it confirms with class B, which allows the irrigation of fruit trees with peeling, roses, ornamental plants, fiber crops (e.g., Flax), berry used for silk production and crops for animal fodder. The characterization of settler effluent showed that average residual concentration of COD, BOD_5 and TSS were 78, 45 and 39 mg L^{-1}, respectively. The maximum removal percentage of COD and BOD_5 reached 78 and 81% respectively. Removal percentage of TSS reached 84%.

Ammonia average removal reached 67% and the organic nitrogen average removal 59%, thus average residual concentration of ammonia and organic nitrogen were 6 and 9 mg L^{-1}. These concentrations do not represent any violation of the national legislation for wastewater reuse; on the contrary, these concentrations could be used as fertilizer in water irrigation.

Effect of seasonal variation on the performance of the integrated system: The treatment system affected by climate variability especially temperature. Figure 3 and 4 showed that the temperature increases during summer season the FP removal efficiency of COD and TSS reached 72 and 75%, respectively. Also, the HRAP showed increase in algae production this is cleared by the increasing percentage in TSS and COD which reached its maximum in summer 157 and 149% more than FP effluent concentration. These results were compatible with the results concluded by Craggs *et al.*[32]. During winter, these percentage decreased by more than 50%. It is also cleared that the algal settleability improved with temperature raising as the tube settler showed better removal percentage during spring and summer more than in winter.

Estimation of coliform group in the ponds and final effluent at different seasons: Oxidation ponds are able to remove pathogens, there are numerous factors which are essential for decreasing bacterial count including, time, temperature, pH, light intensity and dissolved oxygen concentration[33,34].

Results illustrated in Fig. 5 and 6 showed a clear evidence of coliform group removal in warm season is higher than in cold season. The average residual count of fecal coliform in cold season was about 1 log but in summer it is less than one log. Nelson *et al.*[35] demonstrated that the sunlight exposure in algal ponds can led to the maximum removal of coliform bacteria.

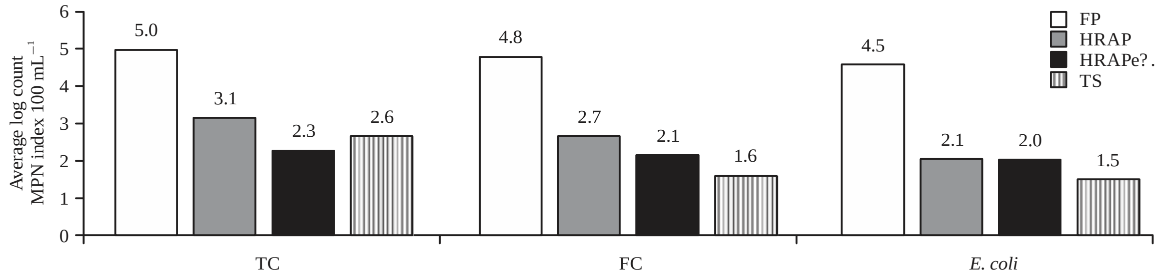

Fig. 5: Average log counts of coliform group in cold season

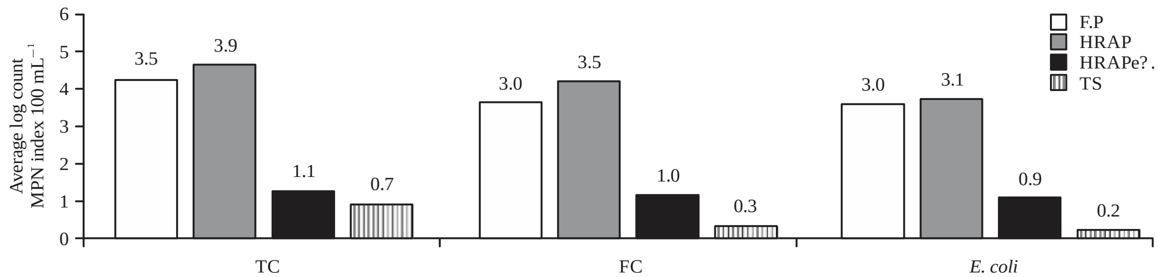

Fig. 6: Average log counts of coliform group in warm season

Table 2: Community structure and species dominancy in HRAP

Algal taxa	HRAP (mixed liquor)	TS effluent
Chlorophyta		
Ankistrodesmus acicularis	+	-
Chlamydomonus variabills	+++	++
Coelastrum sp.	+	-
Dictyosphaerium pulchellum	+	-
Micractinium pusillum	+	-
Scenedesmus obliquus	++++	++++
Odogonium sp.	++	+
Euglenophyta		
Euglena variabilis	+	-
Haematococcus pluvialis	+	-
Cyanophyta		
Oscillatoria limnetica	+++	±
Bacillariophyta		
Cyclotella comta	±	-
Nitzschia linearis	+	-
Stephanodiscus dubius	+	-

++++: Dominant, +++: Plenty, ++: Many, +: Appreciable, ±: Rare, -: Not detected

These results were in compatible with Martinez *et al.*[36], who found that the algal ponds can eliminate total coliform, fecal enterococci and others. In summer the algal activity increases which led to increase in pH and DO which led to pathogen removal[37] confirms that it is not required to reach 9.0 and 9.3 pH value to cause rapid pathogen decay as suggested by other researchers[38]. Pathogen removal took place due to intensity of the solar radiation and high oxygen concentrations that able to damage fecal bacteria indicator. The high removal of fecal coliform bacteria in algal pond unit could also be a result of receiving direct sunlight which leads to prolonged exposure to ultraviolet (UV) radiation[39].

Algal community structure in high rate algal pond

Microscopical examination: Microscopical examination at the initial operation of the HRAP proved that there are diversity of the algal species including *Scenedesmus obliquus*, *Micractinium pusillum*, *Dictyosphaerium pulchellum*, *Coelastrum* sp. After 1 year, continuous operations of the HRAP the dominant algal species were *Scenedesmus obliquus and Chlamydomonas variabills*, in addition to the presence of other strain which is *Odogonium* sp. Also*, Euglenophyta* and *Bacillariophyta* were detected in HRAP. The dominant algal species in the settler effluent was *Scenedesmus obliquus* followed by *Chlamydomonas variabills* and *Oscillatoria limnetica* (blue-green algae) but, *Euglenophyta* and *Bacillario phyta* were not detected (Table 2).

Measurement of algal growth: The growth of community structure was followed up by measuring chlorophyll a content (Fig. 7) in HRAP liquor and tube settler effluent. The maximum log readings of the HRAP mixed liquor reached 3.6 (4941.1) μg L^{-1} were detected during warm season, while the final effluents reached 2.5 (235.5) μg L^{-1}, respectively. The chlorophyll a reading showed its lowest values during winter as it reached 2.9 (765) and 2.2 (180.3) μg L^{-1} for HRAP mixed liquor and tube settler effluent, respectively. This study discover that the readings in the final effluent were lower, this is normal condition due to the settling of the algal cells inside the settler, as the settler separate more than 50% of algal biomass and there is almost no light transfer to the settler.

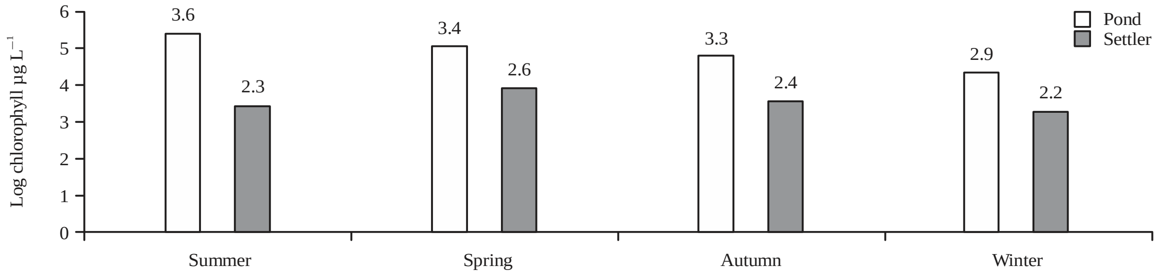

Fig. 7: Log chlorophyll a content during different seasons

Table 3: Fatty acids profile of biodiesel from algal biomass

Fatty acids	Common name	Mass fraction (%)
C:10:0	Capric acid	1.33
C:12:0	Lauric	2.74
C:14:0	Myristic	3.10
C:16:0	Palmitic	28.70
C:18:0	Stearic acid	5.60
C:16:1	Palmitoleic	1.30
C:16:2	-	2.20
C:18:1	Oleic	15.10
C:18:2	Linoleic	5.40
C:18:3	Linolenic	13.50
C:20:1	-	3.20
C:22:0	-	1.30
C:24:0	-	1.70
Total fatty acids (%)		85.17
Saturated fatty acids (%)		44.50
Unsaturated fatty acids (%)		40.67

Algal biomass: The results showed that the biomass in the cold season ranged from 0.2±0.1 to 0.34±0.3 g L^{-1}/day, while the biomass at the warm season ranged from 0.6±0.5 to 1.2±0.54 g L^{-1}/day. These results indicated that the biomass in warm condition is slightly higher than that at cold conditions. These results were similar to those reported by Sheehan *et al.*[40] and Pulz[41], who stated that algal productivity increases with increasing temperature. Settling techniques using tube settler was found to be the most appropriate technology, since it gives about 60% of algal biomass which equivalent to an average of 0.27±0.34 g L^{-1}/day. The average of biomass production in the whole study period was 27 g m^{-2}/day with maximum chlorophyll 3.7 mg L^{-1}. These results were lower than that detected by Vargas e Silva and Monteggia[42], who found that the biomass produced from the HRAP reached 47.1 g m^{-2}/day with maximum chlorophyll 7735 mg L^{-1}. The results were in harmony with that of Park and Craggs[9], who stated that the maximum biomass productivity was (24.7 g m^{-2}/day). Wolkers *et al.*[43] stated that consider algal biomass is composed of 40% oil, 50% protein and 10% sugars. Part of the oil will be used for the production of biofuels and the other part serves as raw material for the chemical industry. Water soluble proteins can be used in food instead of soy protein and partly in cattle feed. Also, the sugars have numerous applications. In addition, the O_2 produced by algae and the removal of nutrients from residual streams generate income.

Carotenoid content of algal biomass: Carotenoid content of algal biomass after oil extraction was 48.839 µg g^{-1}. So the algal biomass can not only be a source for biofuel but also a source of carotenoids which can be used as antioxidants, in addition to colorants agents which can be used in the food industries and several pharmaceutical applications[44-46]. Production of carotenoids using microbiological routes gained scientific and commercial importance within the alimentary and aquaculture fields[47], especially in view of environmental and health awareness by consumers.

Total lipid content and GC analysis of fatty acids methyl esters: The percentage of total lipid for samples collected from HRAP reached 6%±0.5. The fractions of oil for the samples collected from settler were investigated to determine its quality for biofuel production.

The results of FAME of algal oil showed that Palmitic acid percentage (C16:0) reached 27.4% followed by Stearic acid was 5.1%. Since palmitic acid was found in significant percentage and polyunsaturated fatty acids were not detected this is an indication for the possibility of using this oil as a source of biodiesel[48]. The FAME obtained from algal biomass has a brownish yellow color. The content of the fatty acid methyl ester in the biodiesel was 85.17% (Table 3). It is noted from the results that biodiesel from the HRAP contains a major proportion of saturated fatty acid methyl esters.

Fuel properties after blending with gasoline (10%): Biodiesel is a clean burning, environmentally-friendly alternative fuel that could be produced from domestic resources like algae. The blending with petro diesel will reduce sulfur emission, particulate matters and greenhouse gas emissions. It will also insure the security of energy supply and provide the

Table 4: Analysis of biodiesel blended with gasoline (10%)

Experiment	Method	Gasoline	B (10%)
Kinematic viscosity (cSt, 40°C)	ASTM D-445	0.4-0.8	0.4974
Pour point (°C)	ASTM D-97	-40	<-45.0
Density at 15.56	ASTMD-1298	0.7-0.77	0.7386
Cetane index	ASTM D-976	5-20	16.8
Flash point (°C)	ASTMD-93	-45	<-18.0

Table 5: Summary of economic analysis

Item	Cost
Total capital investment (TCI)	63,975,348
Total manufacturing cost (TMC)	732,086,197
Products	
Biodiesel	12,350,000
Cake	71,788,994
Glycerol	1,155,840
Jojoba/carotenoids	991,964,704
total selling cost	1,077,259,539
Net annual profit (NP)	345,173,342
Return on investment (ROI%)	539.541
Pay-back period (years)	0.185

development of jobs at rural communities. Biodiesel can be used in its neat form (B100) or blended at any level with petrodiesel to create a blend. Blends are denoted as "BXX", where "XX" represents the biodiesel fraction (i.e., B20 is 20% biodiesel and 80% petrodiesel)[25].

Engine tests recommended using blends with diesel oil up[49,50] to 20%. Algal biodiesel and diesel oil blends were tested on a diesel engine compared to diesel fuel decreases in thermal efficiency and increases in specific fuel consumption for biodiesel blends were shown[51]. So, algal biodiesel blend B10 (with petrol or gasoline) properties were analyzed at the Egyptian Petroleum Research Institute, Cairo, Egypt according to standard test procedures. The measured physical and chemical properties like density, viscosity, flash point and cetane index (Table 4).

Economic analysis: One aspect to consider when discussing biofuel production from algae is economic cost. In a previous study[48], it was estimated that the cost of biofuel production from oxidation ponds was estimated as $ 1,235/ton. To reduce this cost, it was found that the use of biomass of algae in the production of other compounds of economic value such as carotenoids may contribute to reducing the economic value. Carotenoids extraction was found to cost 4500$/ton. The extracted microalgae cake and produced glycerol are considered as byproducts for the process. The total products' sales cost equals to $1,077,259,539 and the net profit will be the result of subtracting TMC from total sales' cost (Table 5). The assessment performed in this work is classified as a "Study estimate" with a range of expected accuracy[52] from +30% to -20%. While the results of such a study will likely not reflect the final cost of constructing a chemical plant, the technique is useful for providing a relative means to compare competing processes.

CONCLUSION

Treatment of municipal wastewater using the integrated system FP+HRAP+TS approved its high treatment efficiency, because of its simplicity, low construction, maintenance and operation cost.

Tube settler proved to be good technology for harvesting algal biomass and separating algae from the HRAP effluent which reached 60% and it capable of removing 99% of pathogen.

Blending of biodiesel produced from HRAP may contribute to reduce using high amounts of petroleum diesel, hence reduce emissions.

SIGNIFICANCE STATEMENT

This study revealed that, HRAP plays an important role to improve the efficacy of wastewater treatment. The final treated wastewater can be used in many different agricultural applications. In addition, this system can serve the community through the increase of algal biomass that can be used in production of biodiesel and byproducts.

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