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The Differential Efficiency of Chlorella vulgaris and Oscillatoria sp. to Treat the Municipal Wastewater

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Abstract
The utilization of microalgae to treat wastewater becomes an alternative biological wastewater treatment technique worldwide because of its low cost and environmental clean. *Chlorella vulgaris* and *Oscillatoria* sp. were cultured in municipal wastewater under controlled laboratory conditions with continuous light illumination and aeration. Physical properties (pH, TDS and Salinity), and nutrient contents (ammonia, nitrite and total phosphorus)were measured in the raw wastewater. Growth rates of the cultured species in terms of optical densities and cell counts, nutrient salts removal efficiencies were measured during the experimental durations after; 24, 48, 72, 96 , 120 and 144hrs. Lipids, proteins and carbohydrates contents were also evaluated after 144hrs. The recorded optical density of *Chlorella vulgaris* was 0.188±0.01 at 680 nm and 0.17±0.01 at 750 nm after 24hrs then increased to 1.45±0.002 at 680nm and 1.43±0.01 at 750nm at the end of 120hrs, meanwhile the optical density of *Oscillatoria* sp. was increased from 0.132±0.01 at 680 nm and 0.102±0.01 at 750 nm after 24hrs to 1.054±0.004 at 680 nm and 0.99±0.002 at 750 nm at the end of 120hrs of the experiment. On the other hand, the cell count of *Chlorella vulgaris* was enriched from 6.8±2.2 cell/ml after 24hrs to 720±120 cell/ml at the end of 120h of the culturing. Overall, efficiencies of nutrients removal were 99.426%, 100% and 82.211% for ammonia, nitrite and total phosphorus respectively at the end of 120hrs of *Chlorella vulgaris* culturing, while *Oscillatoria* sp nutrient removal efficiencies recorded 98.125%, 100% and 84.718% for ammonia, Nitrite and total phosphorus respectively. *Chlorella vulgaris* biomass was estimated 26.66±7.5% lipid 35.1±1.6% protein and 29.34±3.25% carbohydrate, however *Oscillatoria* sp. biomass was estimated 11.76±0.79% lipids, 32.9±1.92% protein and 27.36±3.78% carbohydrates.
Keywords: municipal Wastewater, *Chlorella vulgaris*, *Oscillatoria* sp., nutrient removal, biochemical parameters.

1. Introduction

Wastewater treatment is applied in many countries to compensate for freshwater shortages. Various conventional methods are used to achieve that goal, but they are costly, a bit harmful and not economic. The Biological wastewater treatments using microalgae were widely accepted as eco-friendly, effective and low cost conventional treatment techniques (Mulbry et al., 2008). These techniques were considered the best solutions to solve the high pollution rates, nutrient enrichment and the dissolved oxygen depletion in addition to producing energy from the algal biomass. Also, microalgae provide a pathway to remove the vital nutrients (nitrogen and phosphorus), carbon dioxide, heavy metals and pathogens from the different types of wastewaters as they necessary for the algal growth(Brennan and Owende, 2010).The bioremediation processes are depending on the ability of microalgae to convert solar energy into useful biomasses by consuming nutrients as phosphorus and nitrogenin the process of photosynthesis (De la Nouë and De Pauw1988; Bolan et al., 2004; Munoz and Guieyssea, 2006).In the bioremediation processes, microalgae consume high amount of nutrients (phosphorus and nitrogen) for the synthesis of 45–60% proteins of the microalgae dry weight, nucleic acids and phospholipids (Oswald, 2012). Nutrient removal can also be achieved by NH_3 stripping and phosphorus precipitating due to the rising in the pH associated with photosynthesis. The harvested algal biomass can be potentially used as an energy source, animals feeding and as a fertilizer (Vilchez et al., 1997; Mulbry et al., 2006). It is estimated that there are 2×10^5 to 10×10^5 different microalga strains in nature, but only about 3×10^4 strains have been described (Sheehan et al., 1988). Among the strains, *Chlorella vulgaris* is known as one of the fastest growing unicellular microalgae, and typically includes 14%-22% of lipid, 51%-58% of protein, 12%-17% of carbohydrates, and 4%-5% of nucleic acid (Becker, 1994). *Chlorella vulgaris* has been reported to be able to readily uptake nitrogen from ammonium ion and ammonia and phosphorus through the cell membrane from the wastewater (Shi et al., 2007; Kassim et al., 2002; Gonzalez et al., 1997;Bich et al., 1999). Many studies demonstrated the remarkable potential of *C. vulgaris* in fixating up to 74% carbon dioxide when grown in a photobioreactor and in absorbing 45–97% nitrogen,28–96 % phosphorus and in reducing the chemical oxygen demand (COD) by 61–86% from different types of wastewater such as; textile, sewage, municipal, agricultural and industrial (Keffer and Kleinheinz, 2002; Aslan and Kapdan, 2006;Yun et al., 1997).Thus, a faster growth rate accompanied by an elimination of wastewater contamination level is a promising and advantageous process. Thus, *Chlorella vulgaris* is considered as one of the best microalga for bioremediation of wastewater with an impressive potential to completely remove ammonium and sometimes modest potential to eliminate phosphorus present in the medium (Gonzalez et al., 1997).The other microalgae species (*Oscillatoria* sp.) is filamentous blue-green microalgae, morphologically consists of small isolated filaments. (Craggs et al., 1997) reached to 100% nutrient (nitrogen and phosphorus) removal from the wastewater by *Oscillatoria* sp.

In the last three decades, Egypt suffers from significant shortages in freshwater resources due to the declining of water lever in the Nile River income. With the increasing number of inhabitants of new cities along the Red Sea; gulfs of Suez and Aqaba; and the northern coast along the Mediterranean, the need for alternative sources of freshwater has become crucial. The most prevalent source of freshwater at these coastal cities has been the desalination of seawater. Along the Red Sea and the gulfs, as well as the Egyptian northern coast along the Mediterranean, more than 150 desalination stations are found producing about 8 million cubic meters daily of freshwater. Consequently, the resultant municipal wastewater from these coastal settlements and its populations are reaching about 5 millions of cubic meters daily, causing intensive environmental hazards to the neighboring marine and terrestrial environments. Subsequently, this research aims to convert the municipal wastewater to a source of freshwater edible for agriculture and, to minimize the environmental risk on the surrounding coastal environment using the biological treatment by *Chlorella vulgaris* and *Oscillatoria* sp.

2. Materials and methods:

2.1. Preparation of Water samples

The raw wastewater samples were collected from Hurghada on the northern Egyptian the Red Sea coast. Samples were filtered through 45µ filter membrane to remove the suspended particles and any materials (≥45µ), then sterilized in the autoclave at 121° C for 20 minutes to avoid any contaminations by the microbial organisms (fungal and bacterial).

2.2. Culture of algae:

Two species of microalga; the unicellular *Chlorella vulgaris* and filamentous *Oscillatoria* sp. were cultured separately in 1000 mL flasks using two different types of nutritive media. The cultures (alga species in nutritive media) were incubated at 25±1ºC and illuminated with white fluorescent lamps at an intensity of 100 µmol photons m^{-2} s^{-1}. The algal culturing flasks were supplied with dry air (Lorenzen, 1964) to provideCO_2 for the photosynthesis and toprevent the settling of the cells at the bottom of the flasks as well as to maintain the algae in suspension without mechanical stress (Persoone et al., 1980).

2.3. Experimental applications

2.3.1. Physicochemical characteristics of wastewater samples

The selected microalga species; *Chlorella vulgaris* and *Oscillatoria* sp. were inoculated in the experimental medium (10% cultured alga/90% raw wastewater) in Erlenmeyer flasks, then illuminated and aerated for five time periods (24hrs, 48hrs, 72hrs, 96hrs and 120hrs). After each period, 100ml of each cultured samples were centrifuged and the supernatants were taken for measuring the physicochemical parameters (dissolved oxygen, TDS, salinity, Sp. conductivity and pH) using YSI 5913**,** however nutrient salts; ammonia, nitrites and total phosphorus were measured spectrophotometrically at 630nm, 540nm and 880nm respectively (APHA, 1995) using JENWAY 6800UV/VIS. The percentage of nutrients removal was calculated by the following equation:

$$\text{Removal Effeciancy (\%)} = {(\text{Initial concentration} - \text{Final concentration})}/{(\text{Initial concentration})}\ \text{x}\ 100$$

To determine the cell counts of *Chlorella vulgaris* and to measure the optical density of *Chlorella vulgaris* and *Oscillatoria* sp., 10ml of each of cultured samples was packed in Neubauer hemocytometer then investigated using spectrophotometer (JENWAY 6800UV/VIS) at 680 and 750 nm. The algal biomass was harvested by centrifugation at 5000 rpm for 10 min at the end of fifth days of cultivation period. The biomass was dried at 60°C in a hot air oven until obtaining a constant weight. The dried biomass of microalgae was further grinded with the help of mortar and pestle into powdered form for its biochemical estimation.

2.3.2. Estimation of lipids, protein and Carbohydrates:

The biochemical composition of resultant algal biomass was analyzed via standard procedures described in (Pádua et al., 2004); lipid by method of (Bligh and Dyer 1959), protein using (Lowry *et al.,* 1951) and carbohydrate contents by the method of (Dubois *et al.,* 1956) .

3. Result and Discussion

3.1. Wastewater quality

Dark brown color, with un-acceptable odors, slight alkaline (pH~9.08) and reducing nature (E.C.~2.67mmohs./cm) as indicated by the dissolved oxygen content (0.6mg/l) were observed in the collected samples. The measured total dissolved salts (TDS) and salinity (‰) recorded 1508mg/l and 1.17‰ respectively. The nutrient salts of row wastewater before algal treatment showed significantly high contents for ammonia and total phosphorus (69.283, and 41.15 mg/l respectively), meanwhile nitrites showed low value 0.0384mg/l due to the continuous aeration at the treatment station (Table, 1).

3.2. Experimental duration and physical changes

Microalgae growth is affected by the availability of nutrients and pH (Azov and Shelef, 1987), light intensity, temperature (Poulio and De la Nouë, 1986) and many other biological factors as the high initial algal density (Lau et al., 1995) whereas, the increasing in initial algal density leads to high nutrients removal. However, the high algal density would lead to self-shading, accumulation of auto-inhibitors, and reduction in photosynthetic efficiency (Fogg, 1975; Darley, 1982).

The recorded optical density of *Chlorella vulgaris* was 0.188±0.01 at 680nm and 0.17±0.01 at 750nm after 24hrs and then increased to 1.45±0.002 at 680nm and 1.43±0.01 at 750nm at the end of fifth day (Table 2,3; Fig. 1,2). The optical density of *Oscillatoria* sp. was enriched from 0.132±0.01 at 680nm and 0.102±0.01 at 750nm after 24hrs to 1.054±0.004 at 680nm and 0.99±0.002 at 750nm at the end of fifth day of the experiment. On the other hand, the cell count of *Chlorella vulgaris* was enriched from 6.8±2.2 cell/ml after 24hrs to 720±120cell/ml at the end of fifth day of the culturing. After 144hrs the optical densities of both species and the cell count of *Chlorella vulgaris* were regressed obviously (Fig. 1, 2) due to the nutrients depletion in the surrounding aqueous media. (Becker, 1994) attributed the declining of the cultured microalgae to nutrient depletions and the pH variations. It is obvious that growth rate of cultured microalgae and bioremediation efficiency of wastewater were significantly affected by the pH. (Karin, 2006) documented that the increasing of dissolved oxygen and pH in wastewater are causing phosphorus precipitation as well as ammonia and hydrogen sulfide removal. During the period of the experiment, pH values were significantly decline from 9.08 before *Chlorella vulgaris* aquaculture to 8.16 at the end of fifth day of culturing and from 9.08before *Oscillatoria* sp. aquaculture to 8.24 at the end of fifth day of culturing (Table 4). (Aarti et al., 2008) and (Fathi et al., 2013) attributed the significant deceasing in pH to the increasing in the photosynthetic activities with intensive flourishing of the cultured species. According to (Mostafa 2010), variation in pH values can affect the metabolism and algal growth by altering the equilibrium of inorganic carbon (C), changing availability of nutrients in addition to affecting the cell physiology. In the same manner, slight decreasing in the TDS was observed in the two species throughout the period of the experiment. In *Chlorella vulgaris* culturing flask, the TDS was decreased from 1508µg/l in the row wastewater to 1430 µg/l at the end of fifth day and in *Oscillatoria sp.*, TDS was declined from 1508µg/l to1306.5µg/l at the end of fifth day of the experiment (Table 4). (Nanda et al., 2010) and (Azarpira et al., 2014) found a unique mechanism of microalgae bioabsorption/adsorption of different types of dissolved solids from the wastewater that may be responsible for reducing TDS to the lowest level. Moreover, (Rao et al., 2011) and (Ahmad et al., 2013) stated that the reduction in TDS in the wastewater was attributed to the utilization of various inorganic nutrients by microalgae.

3.3. Nutrient salts

A- Ammonia depletion

Throughout the period of the experiment, ammonia contents were declined from 69.283±1.27 mg/l before treatment to 0.397±0.069mg/l at the end of fifth day of the experiment with removal efficiency reached 99.426% by *Chlorella vulgaris*, meanwhile it was decreased from 69.283±1.27 mg/l before treatment to 1.298±0.198 mg/l at the end of fifth day of the experiment with removal efficiency reached 98.125 % by *Oscillatoria* sp. (Fig. 3; table 5). Nitrogen is considered the second most important nutrient to microalgae after carbon since it may comprise more than 10% of the biomass (Becker, 1994). The bioavailable nitrogen was existed in many forms; ammonium (NH_4^+)and nitrate (NO_3^-) that are the most common nitrogen compounds assimilated by microalgae (Oliver and Ganf, 2000). In addition to these compounds, urea ($CO(NH_2)_2$) and nitrite (NO_2^-) were widely used as nitrogen sources. The preferred compound as nitrogen source to the microalgae was ammonium, and when this is available, no alternative nitrogen sources will be consumed (Bhaya et al., 2000). However, ammonium concentrations higher than 20mg NH_4^+-N per liter are not recommended due to ammonium toxicity (Borowitzka, 1998), also, the toxicity of nitrite at higher concentrations makes it less convenient (Becker, 1994). (Rao *et al.* 2011) found that *Chlorella vulgaris* has the ability to consume all forms of nitrogen substantially and particularly ammonia and nitrates. They revealed that ammonia removal efficiency of *C. vulgaris* was nearly 100 % in the wastewater. (Lau et al., 1996) recorded the removal efficiency by *Chlorella vulgaris* was reached 86%. Meanwhile, (Colak and Kaya 1988) found elimination rates of nitrogen reached about 50.2% in industrial wastewater treatment. (Ruiz et al., 2010); (Wang et al., 2010); (Khan and Yoshida, 2008) and (Gonzalez and Canizares, 1997) reported nitrogen removal efficiency of *Chlorella* sp. Was 23–100%. On the other hand, (Craggs et al., 1997) recorded ammonia removal efficiency 100% within *Oscillatoria sp.* in municipal wastewater

B- Nitrite depletion

During the experimental duration; nitrite concentration was decreased from 0.038±0.002 mg/l before treatment to 0.00mg/l at the end of fifth day with removal efficiency of 100% by both species *Chlorella vulgaris* and *Oscillatoria* sp. which could probably result from the nitrification activity (Fig. 3; table 5). It is important to notice that nitrite concentration was significantly low in the raw wastewater due to the aeration process.

Nitrate and nitrite undergo reduction with the assistance of nitrate reductase and nitrite reductase, respectively. Nitrate reductase uses the reduced form of nicotinamide adenine dinucleotide (NADH) to convert nitrate into nitrite. Then, nitrite is reduced to ammonium by nitrite reductase and ferredoxin (Fd) (Cole and Brown, 1980). Thus, all forms of inorganic nitrogen are ultimately reduced to ammonium prior to incorporate into amino acids within the intracellular fluid.

C-Total Phosphorus depletion

The obtained results of the experiment showed that total phosphorous concentration was reduced from 41.15±2.32mg/l before treatment to 7.266±0.1107 mg/l at the end of fifth day with removal efficiency of about 82.21% by *Chlorella vulgaris and* from 41.15±2.32 mg/l before treatment to 6.288±0.0112mg/l at the end of fifth day with removal efficiency of about 84.71% by *Oscillatoria* sp. (Fig. 3; table 5). Phosphate removal by microalgae during phytoremediation is due to the utilization of phosphorus for growth. Phosphorus is another essential macro-nutrient for algal growth consumed as inorganic orthophosphates (PO_4^{3-}). The uptake of orthophosphate by algae is an active process that requires energy. Organic phosphates can be converted to orthophosphates by phosphatases at the cell surface, and this occurs especially when inorganic phosphate is in short supply (Fogg, 1975). Microalgae are able to assimilate phosphorus in excess then stored within the cells in the form of polyphosphate (volutin) granules. These reserves can be sufficient for prolonged growth in the absence of available phosphorus (Fogg, 1975) and (Oliver and Ganf, 2000).

(Lau et al., 1996) found removal efficiency of about 78% in *Chlorella vulgaris*, meanwhile (Colak and Kaya 1988) found that the removal efficiency of phosphorus was reached (85.7%) in industrial wastewater and (97.8%) in domestic wastewater treated by algae. In the same manner, (Ruiz et al., 2010), (Wang et al., 2010), (Khan and Yoshida, 2008) and (Gonzalez and Canizares, 1997) found the removal efficiencies between 20–100% in *Chlorella* sp. (Craggs et al., 1997) recorded phosphorous removal efficiency 100% within *Oscillatoria* sp. in the municipal wastewater. It is obvious that the measured nutrients were decreased significantly due to the fast assimilation by algae after 72hrs followed by slight declining during the rest of the experimental period. So we suggest that restricting the experiment within 72hrs is enough to achieve maximum nutrients reduction when using these strains under the current conditions. Table (6) showed different removal efficiencies of total nitrogen (TN) and total phosphorous (TP) for different microalgae species under different laboratory conditions by many authors worldwide.

The most in impressive notice was the slight increasing of the nutrient salts (ammonia, nitrite and total phosphorus) in the experimental culturing media after passing the 120hrs of the experiment due to the release of cellular nutrients, suggesting that a retention time of 3 days is enough to achieve maximum nutrient reductions when using these strains under the current settings of the cultured microalgae (table 5).

3.4. Biochemical composition of tested algae:

Total lipid, total protein and total carbohydrate content were measured after five days of cultivation in wastewater. Microalgal lipid production is central in many research projects aiming to find sustainable means of producing liquid fuels (Konur O., 2011).The experimental data obtained during this study showed that, the lipid content of *Chlorella vulgaris* was 26.66 ± 7.5 % of biomass dry weight. While the lipid content of *Oscillatoria sp*. was 11.76±0.79% of biomass dry weight (Fig 4). Similar results were found by (Farooq et al., 2013) where the total lipid content of C. *vulgaris* was 26.7% and 26% by (Guldhe et al., 2017). It is clear that *Chlorella vulgaris* have shown excellent efficiency for nutrient removal from wastewater but the concentration of algal biomass and lipids content were not satisfactory for biofuel production as maintained by (Deng et al. 2009), (Mata et al. 2009), (Sialve et al. 2009) and (Li et al. 2008). In this respect also (Sialve et al., 2009) reported that the accumulation of lipids in cyanobacterial biomass (such as *Oscillatoria sp.*) is fairly low. Furthermore, (Demirbas and Demirbas 2011) found that cyanobacteria are not rich in lipids (up to 20%).

Proteins are of central importance in the chemistry and composition of microalgae. They are involved in capital roles such as growth, repair and maintenance of the cell as well as serving as cellular motors, chemical messengers, regulators of cellular activities and defense against foreign invaders (Solomon et al., 1999). Total protein content in tow investigated alga were 35.1 ± 1.6 % of biomass dry weight for *Chlorella vulgaris* and 32.9±1.92% of biomass dry weight of *Oscillatoria sp.* (Fig 4). Similar results found by (Zayadan et al., 2017) where the total protein content of *Chlorella vulgaris* was 35%. (Balaji et al., 2016) found that total protein content of *Oscillatoria sp*. was lower than this study (Table7). (Ming-Sheng et al., 2016) attributed that, the slight high protein content in *Chlorella vulgaris* due to higher N content in domestic wastewater. Also (El-Enany and Issa, 2000) found that the diluted sewage water produced high protein content. Whereas, the high levels of wastewater concentrations were inhibitory to some Cyanobacteria.

The measured total carbohydrate contents of the two investigated alga were 29.34±3.25% of biomass dry weight for *Chlorella vulgaris* and 27.36±3.78% of biomass dry weight of *Oscillatoria sp*. Similar results found by (Zayadan et al., 2017) where *Chlorella vulgaris* grown on wastewater showed carbohydrate content of 29% but lower than that found by (Guldhe et al. 2017). While *Oscillatoria sp.* grown on Piggery Farm Effluent showed carbohydrate content lower than this study (Wichienprers*,* 2007).Very few studies on microalgal cultivation using wastewaters report the biochemical constituents of the biomass (Table7).

3.5. Conclusion

The study clearly supports the algal use in wastewater treatment whereas, it provided three purposes; suitable source of portable water in agriculture, to maintain a clean environment as well as generation of valuable biomass from algae. Our results demonstrated the efficiency of the microalgae *Chlorella vulgaris* and *Oscillatoria sp.* to remove ammonia, nitrite and total phosphorus from the municipal wastewater. *Chlorella vulgaris* was largely assimilated the NH_3, NO_2 and PO_4 with percentages of 99.408, 100% and 82.211% respectively while *Oscillatoria* sp. removal efficiency was 98.125%, 100% and 84.718% for NH_3, NO_2 and PO_4 respectively. Lipid content in *Chlorella vulgaris* was higher than lipid content of *Oscillatoria sp*., which makes *Chlorella vulgaris* is more convenient in the biofuel production. The experiment was extended for 144hrs with significant nutrient removal after 72hrs then the removal process was decline then after 120hrs the nutrient contents were slightly increased due to the release of cellular nutrients, consequently, the experiment recommended by applying this technique for three days only.

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Table (1): Physicochemical characteristics of row wastewater

Parameter	Concentration
Color	Dark brown
Odor	Un-acceptable
Dissolved oxygen	0.6 mg/l
SPC	2.67
Total Dissolved Solids	1508
Salinity	1.17
PH	9.08
NH_3	69.283±1.2 mg/l
PO_4	41.15 ±2.32 mg/l
NO_2	0.0384±0.002 mg/l

Table (2) growth rate of *Chlorella vulgaris* according to optical density and cell count during five days of cultivation:

Growth		**0**	**24h**	**48h**	**72h**	**96h**	**120h**	**144h**
Optical density	680 nm	0.07 ± 0.006	0.188 ± 0.008	0.85 ± 0.007	0.98 ± 0.002	1.27 ± 0.004	1.45 ± 0.002	1.22±0.007
	750 nm	0.04 ± 0.002	0.17 ± 0.005	0.81 ± 0.002	0.94 ± 0.01	1.24 ± 0.004	1.43 ± 0.007	1.19±0.003
Cell count	(Cell × 10^5 . ml^{-1})	2.5 ± 1.2	6.8 ± 2.2	58 ± 23.5	203.2 ± 58	512 ± 90	720 ± 120	620±90

Table (3) growth rate of *Oscillatoria s*p according to optical density during five days of cultivation:

		0	**24h**	**48h**	**72h**	**96h**	**120h**	**144h**
Optical density	680 nm	0.04 ± 0.007	0.132 ± 0.008	0.242 ± 0.001	0.492 ± 0.002	0.81 ± 0.002	1.054 ± 0.004	0.985±0.002
	750 nm	0.02 ± 0.002	0.102 ± 0.005	0.21 ± 0.004	0.44 ± 0.002	0.74 ± 0.005	0.99 ± 0.002	0.898±0.003

Table (4) the change in physical characteristics of the used wastewater throughout the experimental period:

Time in hrs	D.O		SPC		TDS		salinity		pH	
	Chlorella vulgaris	*Oscillatoria sp*	*Chlorella vulgaris*	*Oscillatoria sp*	*Chlorella vulgaris*	*Oscillatoria sp*	*Chlorella vulgaris*	*Oscillatoria sp*	*Chlorella vulgaris*	*Oscillatoria sp*
24hrs	5.3	3.8	2.37	2.17	1535	1420	1.12	1.13	8.97	8.98
48 hrs	4.2	3.9	2.28	2.19	1476	1423	1.16	1.12	8.78	8.96
72 hrs	5.1	3.3	2.24	2.2	1456	1430	1.14	1.12	8.44	8.84
96 hrs	5.2	3.7	2.12	2.11	1435	1365	1.12	1.07	8.17	8.69
120 hrs	5.1	3.6	2.1	2.01	1430	1307	1.1	1.02	8.16	8.24

Table (5): The removal rates of nutrients by *Chlorella vulgaris* and *Oscillatoria* sp. from the wastewaters during the experiment.

Time in hrs.	***Chlorella vulgaris.***						***Oscillatoria sp.***					
	Ammonia		**Nitrite**		**Total Phosphorus**		**Ammonia**		**Nitrite**		**Total Phosphorus**	
	Conc. (mg l^{-1})	**Removal%**	**Conc. (mg l^{-1})**	**Removal%**	**Conc. (mg l^{-1})**	**Removal %**	**Conc. (mg l^{-1})**	**Removal%**	**Conc. (mg l^{-1})**	**Removal%**	**Conc. (mg l^{-1})**	**Removal%**
24hrs.	61.4±0.6	11.374	0.024±0.0004	35.286	37.52±0.01	8.82	65.71±0.225	5.14	0.0254±0.004	33.854	21.9±0.03	46.78
48 hrs.	9.225±0.025	86.684	0.015±0.0002	59.895	33.1±0.02	19.562	40.5±0.4	41.54	0.0216±0.0003	43.75	9.301±0.0087	77.397
72 hrs.	6.767±0.133	90.242	0.0117±0.001	69.401	8.535±0.185	79.258	5.9±0.3	91.48	0.0173±0.003	54.817	7.17±0.0062	82.55
96 hrs.	0.488±0.044	99.295	0.0049±0.0003	86.979	7.825±0.01	80.984	5.03±0.187	92.73	0.0056±0.0004	85.416	6.922±0.032	83.177
120 hrs.	0.397±0.069	99.426	0	100	7.266±0.110	82.211	1.298±0.198	98.125	0	100	6.288±0.0112	84.718
144hrs	1.047±0.073	-	0.0079±0.00015	-	8.545±0.285	-	1.359±0.226	-	0.0102±0.0027	-	7.56±0.04	-

Table (6) Nutrient removal, biomass productivity and lipid content of various genera of cyanobacteria and microalgae in different waste streams.

Category	Genus and species	Waste stream	Reactor type	Retention time (d)	TN		TP		Biomass productivity (g L^{-1} d^{-1})	Lipid content (% dry cell weight)	References
					Initial conc. (mg L^{-1})	Removal efficiency (%)	Initial conc. (mg L^{-1})	Removal efficiency (%)			
Cyanobacteria	*Oscillatoria sp.*	c	Raceway	14	498	100	76	100	-	-	(Craggs et al., 1997)
	Phormidium Bohneri	c	Phototbioreactor	2-3	12-17	53-62*	3-18	100	0.023-0.057	-	(Laliberte et al., 1997)
	P. bohneri	a	Phototbioreactor	30	0.9-1.1	82*	0.08-0.15	85	-	-	(Dumas et al., 1998)
	Planktothrix Isothrix	c	Phototbioreactor	9	43-59	26-40*	7.5	100	0.027-0.05	-	(Margarita Silva-Benavides and Torzillo, 2012)
	Spirulina sp.	d	Raceway	-	-	84-96*	-	72-87	1.44-1.51	-	(Olguin et al., 2003)
	Spirulina sp.	b	Photobioreactor	28	167	100	20	100	0.1	7.1-16.9	(Markou et al., 2012)
	Spirulina Platensis	b	Raceway	15	2-3	96-100*	18-21	87-99	5.32-7.42	8.1-11.2	(Phang et al., 2000)
	Synechococcus elongatus	e	Phototbioreactor	8	25.5	29-54**	6.7	77-88	-	-	(Aguilar-May and del Pilar Sanchez-Saavedra, 2009) (Garbisu et al., 1992)
Chlorophyte	*Botryococcus braunii,*	c	Flask	10	4.48-7.67	100**	0.04-0.39	100	-	-	(Sawayama et al., 1992)
	B.braunii,	c	Photobioreactor	35	5.5	27.3**	0.08	62.5	0.4	-	(Sawayama et al., 1994)
	Chlorella sp.	c	Photobioreactor	13-21	290	61†	530	61	3.46	4.7–6.3	(Min et al., 2011)
	Chlorella sp.	c	Flask	10	33-71	75-82*	6-201	83-91	-	-	(Wang et al., 2010b)
	Chlorella sp.	d	Flask	21	100-240	76-83	15-30	63-75	-	9-13.7	(Wang et al., 2010a)
	C. vulgaris	a	Flask	30	1074	89.5	180	92	1-1.38	-	(Wang et al., 2010c)
	C. vulgaris	d	Flask	8	1722	93.6	111.6	89.2	0.4-0.76	-	(Heredia-Arroyo et al., 2011)
	C. vulgaris	a	Photobioreactor	9	36.3	90-95*	111.8	10-60	-	-	(Gonzalez et al., 1997)
	C. vulgaris	b	Raceway	12	0.47-50.83	4.4-45.1*	0.07-4.01	33.1-33.3	0.1-0.2	40	(Lim et al., 2010)
	C. kessleri	e	Flask	12	129.6	81.1*	-	-	-	-	(Lee and Lee, 2002)
	C. kessleri	e	Flask	3	168	8-19**	10-12	8-20	-	-	(Lee and Lee, 2001)
	Chlamydomonas reinhardtii	c	Photobioreactor	31	128.6	55.8†	120.6	17.4	2	25.25	(Kong et al., 2010)
	Scenedesmus sp.	e	Photobioreactor	0.2-4.5	14-44	30-100	1.4-6	30-100	-	-	(Zhang et al., 2008)
	Scenedesmus sp.	d	Photobioreactor	5	100	90*	-	-	0.2	-	(Park et al., 2010)
	Scenedesmus sp.	e	Photobioreactor	14	5-15	83-99	0.2-1	99	0.15-0.65	30-53	(Xin et al., 2010b)
	Scenedesmus sp.	c	Flask	15	15.5	98.5	0.5	98	0.11	31-33	(Xin et al., 2010a)
	S. obliquus	c	Photobioreactor	0.2-8	27	79-100*	12	47-98	-	-	(Ruiz-Marin et al., 2010)
	S. obliquus	c	Photobioreactor	8	27.4	79-100*	11.8	55-98	0.024	27-34	(Martınez et al., 2000)

* Ammonia nitrogen (NH_4^+-N), ** Nitrate (NO_3^--N), Nitrite (NO_2^--N), † Total Kjeldahl nitrogen

a. Animal wastewater; b. Industrial wastewater; c. Municipal wastewater; d. AD effluent; e. Artificial wastewater

Table (7): Comparisons for lipids, proteins and carbohydrates measured in the present study with the previous studies:

Algal species	Types of wastewater	Lipid%	Protein %	Carbohydrate %	Ref.
Chlorella vulgaris	Domestic Wastewater	26.66±7.5	35.1±1.6	29.34±3.25	This study
	Dairy wastewater	10.3	-	-	Qin *et al* (2014)
	Brewery wastewater	26.7	-	-	Farooq *et al* (2013)
	Urban wastewater	8.01±0.35	-	-	Singh *et al*(2017)
	synthetic domestic wastewater	23.4-28.5	40.9-50.7	-	Ming-Sheng *et al* (2016)
	aquaculture wastewater	26-39.1	24.57-29.46	32.79-35.97	Guldhe*et al* (2017)
	Domestic Wastewater	30	35	29	Zayadan *et al* (2016)
	artificial wastewater	20–42%	-	-	Feng *et al* (2011)
	Domestic Wastewater	13.20±1.87			MOSTAFA *et al* (2012)
Oscillatoria sp	Domestic Wastewater	11.76±0.79	32.9±1.92	27.36±3.78	This study
	synthetic wastewater	26.89	-	-	Economou*et al* (2015)
	Domestic Wastewater	6.80±0.65	-	-	MOSTAFA *et al* (2012)
	Piggery Farm Effluent	2.7	51.9	24	Wichienprers (2007)
	tannery effluent	-	23±0.18%	-	Balaji*et al* (2016)

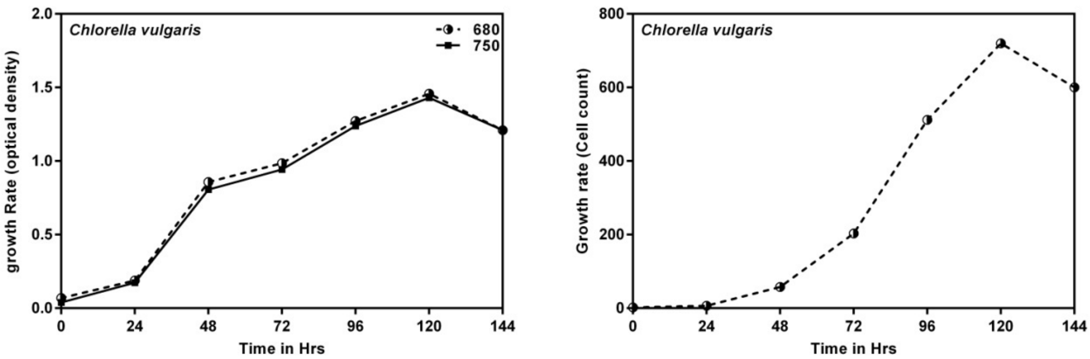

Fig.(1) Growth rate of *Chlorella Vulgaris* according to optical density and cell count during the cultivation period.

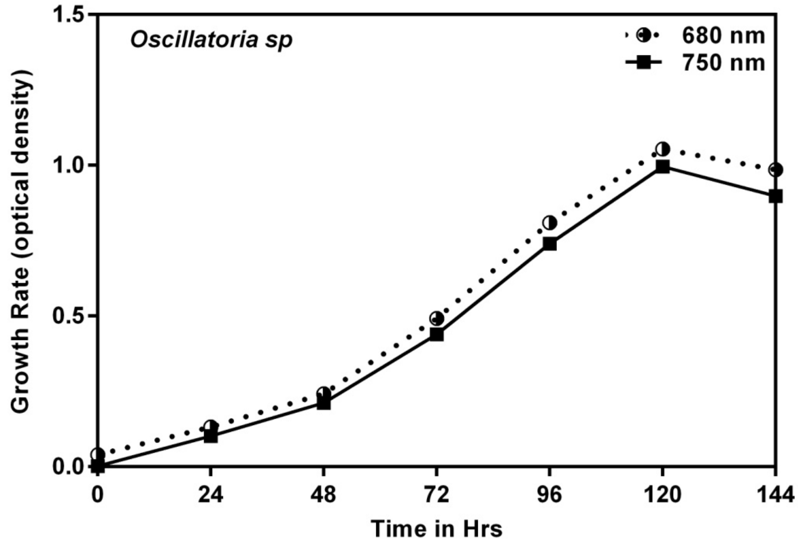

Fig.(2)Growth rate of *Oscillatoria* sp. according to optical density during the cultivation period.

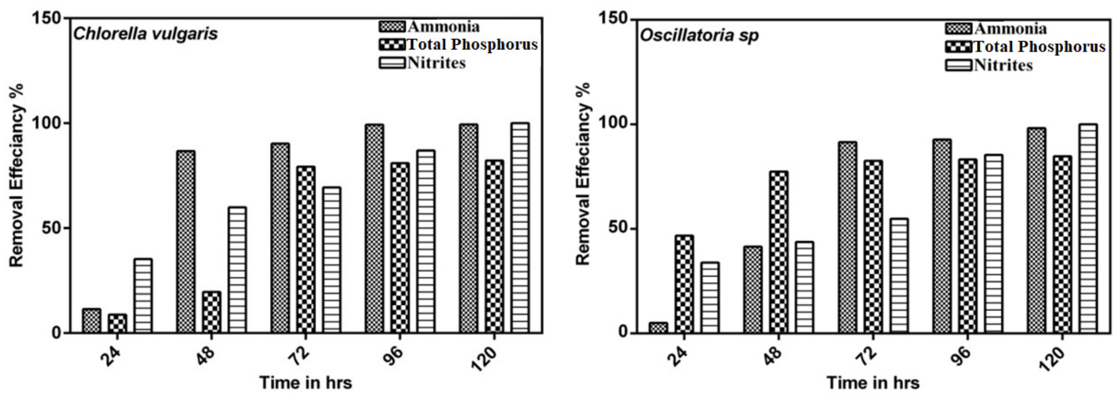

Fig. (3) The removal efficiencies of nutrients in *Chlorella Vulgaris* and *Oscillatoria* sp. wastewater during the experiment.

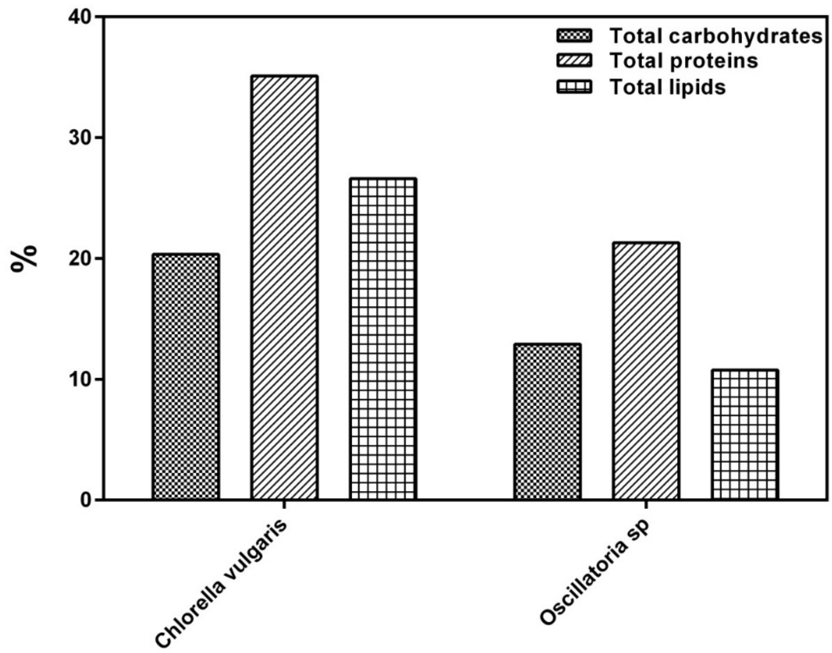

Fig.(4) : The average contents of lipids, proteins and carbohydrates of cultured microalgae after 120hrs of the experiment.