Treatment of Mixed Domestic-industrial Wastewater Using microalgae *Chlorella* sp.

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Abstract: Microalgae culture offers an effective solution for wastewater treatments, because they provide a tertiary biotreatment coupled with the production of potentially valuable biomass, which can be used for different purposes. The present study demonstrated the growth of *Chlorella* sp. in mixed domestic-industrial wastewater without sterilization in a laboratory scale batch process under the continuous illumination of light and continuous aeration and evaluated efficiency of *Chlorella* sp. for eliminating ammonium- nitrogen, nitrate- nitrogen, phosphorus, coliform bacteria, biological oxygen demand (BOD), chemical oxygen demand (COD) and heavy metals. The growth of microalgae *Chlorella* sp. in wastewater was relatively lower than its growth in standard medium (Z-medium) under the same conditions, where dry weight recorded 1.42 and 1.13gm l^{-1}, at the same time chlorophyll (a) recorded 5.65 and 4.55 mg l^{-1}while, cell count was 22.3×10^6 and 12.8×10^6 in z-medium and wastewater respectively. The removal efficiency percentage of BOD, COD, NH_4-N, NO_3–N and PO_4–P reached 90.8 %, 80.1 %, 98.9 %, 87.6 %, and 90 %, respectively. *Chlorella* sp. has the ability to accumulate the heavy metals from the wastewater to Whereas, the heavy metals biosorption performance of *Chlorella* sp. Was higher in accumulating nickel (99.5 %), Mn (73.2 %), Fe (92.2 %), Cu (54.5 %), Zn (51.4), Cr (56.3 %), Mo (99.7 %), Al (98.8 %), Si (48.5 %), V (100 %), Ti (100 %), Sr (41.9 %), Therefore, removal of heavy metals and nutrients by the tested algae is strongly recommended as a powerful technique for the removal of pollutants from wastewater.

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1. Introduction

Rapid industrialization, population growth, and complete disregard for environmental health have led to global environmental pollution **(Dash *et al.*, 2013).** Among all the environmental pollutions, Pollution of water resources is a serious and growing problem **(Karuppaiah *et al.*, 2015).** While major improvements in wastewater treatment have reduced nutrient loading to watersheds from point sources over the last two decades, further reductions are necessary to meet water quality goals and reduce coastal eutrophication **(Filippino *et al,* 2015).** Wastewater treatment is generally divided in three or four main stages, which represent the degree to which the water is treated. These stages are: preliminary treatment, primary treatment, secondary treatment, and tertiary or advanced treatment **(Prescod, 1992).** The conventional methods of wastewater treatment are very costly, consuming energy and producing high quantity of sludge **(Ghosh and Singh, 2005; Abdel Raouf *et al.*, 2012).**

Wastewater treatment by Microalgae has been investigated for over 4 decades as an environmentally sound alternative to remove nutrients and heavy metals from wastewater sources **(Shankar, 2011).** The idea of using microalgae for wastewater treatment originally developed in the 1950s in California by William Oswald **(Oswald and Gotaas, 1957; Oswald, 1963).** The use of macro algae or microalgae for effective removal or biotransformation of pollutants, including nutrients and xenobiotics from wastewater and CO_2 from waste air called phycoremediation **(Olguin, 2003).**

Microalgae are photosynthetic microorganisms which use energy from the sun to grow, consuming inorganic nutrients and CO2, they accumulate organic matter in the form of proteins, lipids, carbohydrates, hydrocarbons and other small molecules and pigments **(Ruiz-Martinez *et al.*, 2012).** They are the major primary producers for organic compounds; and play a central role as the base of the food chain in aquatic systems **(Abdel Raouf *et al.*, 2012).**

Advantages of using algae for wastewater treatment include: low operational cost, a food source for fish or farm animals, avoidance of the sludge handling problem, and direct discharge of oxygenated effluent water into the water bodies **(Choi and Lee, 2012; wang *et al.*, 2013).** Furthermore, nutrients are not only removed from the wastewater, but can also be captured and returned to the terrestrial environment as agricultural fertilizer. Another advantage is photosynthetic CO_2 fixation, which contributes to mitigating greenhouse gases **(Wang *et al.*, 2008; Van den Ende *et al.*, 2012).** Microalgae are able to serve a dual role of bioremediation of wastewater as well as generating biomass for biofuel production **(Mulbry *et al.*, 2008; Fathi *et al.*, 2008).** Usually, algae isolated from a wastewater treatment plant site or real water body can adapt to the practical conditions better and show higher efficiency of inorganic nutrient removal **(Xin *et al.*, 2010).**

Wastewater treatment in Waste Stabilization Ponds (WSPs) is "green treatment" achieved by the mutualistic growth of microalgae and heterotrophic bacteria. The algae produce oxygen from water as a by-product of photosynthesis, this oxygen is used by the bacteria as they aerobically bio-oxidize the organic compounds in the wastewater and produce carbon dioxide which is fixed into cell carbon by the algae during photosynthesis **(Aslan and Kapdan, 2006; Brito *et al.,* 2007; Hodaifa *et al.*, 2010 a, b; Sharma and Khan 2013).** Microalgae assimilate a significant amount of nutrients because they require high amounts of nitrogen and phosphorus for the synthesis of proteins (45-60% of microalgal dry weight), nucleic acids, ATP, and phospholipids **(Oswald, 2003).** The oxygen and pH variation induced by microalgae photosynthesis help reduce coliform and other pathogenic bacteria in the effluent **(Metcalf and Eddy, 2003; Kiso *et al.*, 2005).**

Most heavy metals are well-known toxic and carcinogenic agents and when discharged into the wastewater represent a serious threat to the human population and the fauna and flora the receiving water bodies **(Monika *et al.*, 2014).**

The algae have many features that make them ideal candidates for the selective removal and reducing concentration of heavy metals which include high biosorption capacity, high tolerance to heavy metals, ability to grow both autotrophically and heterotrophically, large surface area/volume ratios, phototaxy, phytochelatin production and its potential for genetic manipulation **(Chekroun and Baghour, 2013; Kumar *et al.*, 2015).** It is well established that several marine and fresh water algae are able to take up various heavy metals selectively from aqueous media and to accumulate these metals within their cells **(Afkar *et al.*, 2010; Kumar and Gaur, 2011; Chen *et al.*, 2012).** There are several reports that different species of several fresh water microalgae like *Chlorella sp., Anabaena sp., Westiellopsis sp., Stigeoclonium sp., Synecococcus sp.* etc. have high tolerant capacity for various heavy metals **(Dwivedi, 2012).** The main key points in this research is selecting the proper microalgae species able to grow in the mixed domestic-industrial wastewater and study the effect of wastewater on its growth, then evaluate its ability to remove inorganic nutrients as (ammonium, nitrate, phosphate), biological oxygen demand (BOD), chemical oxygen demand (COD), coliform bacteria also the removal of different heavy metals as Ni, Mn, Fe, Cu, Zn, Cr, Mo, Al, Si, V, Ti, Sr.

2. Materials and Methods

Organism and Culture Condition

The selected microalgal species (*Chlorella* sp.) was isolated from El Alalma wastewater treatment station in Beni-Suef city, Egypt. Isolation and purification were made by dilution and plating out method as described by **Hilary and Erica (1982).** The alga was grown in Z-medium which described by **Staub, (1961)** and incubated at 30 ± 2°C with continuous light intensity of 4000 lux and continuous aeration.

Wastewater Collection

The wastewaters used in the study, were collected (in June 2014) from El Alalma wastewater treatment station situated in Beni-Suef city, Egypt, Which receives wastewater from domestic and industrial effluents.

Determination of microalgal growth

Growth of *Chlorella* sp. was determined by the different methods (cell count, chlorophyll content, dry weight).

A- Cell Count

The laboratory technique of phytoplankton counting developed by **Utermohl (1936 & 1958)** was applied for quantitative elaboration. The liquid algal culture was swirled to make a homogenous suspension and the containing was conducted using an improved Neubauer haemocytometer.

B-Chlorophyll content

Total chlorophyll content was determined according to the method described by **Strickland and Persons (1972).** A definite volume of well-shacked culture sample was filtered through glass fiber (Satorius, SM 13400). Then homogenized in 80 % acetone and kept in freezer for about 24h, to ensure complete extraction. The extract was diluted to a definite volume (25 ml). After10 min centrifugation (5000 rpm), one ml of the chlorophyll extract was used for the determination of chlorophyll (*a*)

C-Dry weight

Samples of 100 ml of the selected algal species were harvested periodically at different time intervals (every two days) to the end of the experiment (20 days), then centrifugated in centrifuge (3000 rpm) then the algal residue were washed three times by saline water then dried in an oven at 60 to 70 **°C** to obtain a constant weight. Samples were cooled in desiccators for 30 min before dry weight measured.

Experimental set up

In the present investigation, domestic-industrial wastewater was used after the preliminary sieving step in the wastewater treatment station to git rid of the large suspended solids. 3 measuring flasks with 6 liters capacity were used for the treatment of wastewater. The 1st flask contain 3 liters of Z-medium inoculated with *chlorella* sp., the 2nd flask contain 3 liters of wastewater inoculated with *chlorella* sp. The 3rd contains 3 liters of wastewater without inoculation was left as control; the 3 flasks were incubated at 30 ± 2°C with continuous light intensity of 4000 lux. and continuous aeration.

Physico-Chemical Characteristics of Wastewater

pH value, total suspended solids (T.S.S) and electrical conductivity (EC) of wastewater was measured as following, pH of the wastewater sample was determined 2 days interval to the end of the treatment period (20 days)), in addition to zero time using pH meter model (191WIW, Germany). EC and T.S.S were determined at zero time using conductivity meter model (191WIW), Germany.

Estimation of total coliforms

Wastewater (mixed domestic-industrial) samples were collected for the experiment was serially diluted (ten fold dilution) in sterile .85 % physiological saline solution. Total coliforms were enumerated from 0.1 ml sample spread on to the surface of autoclaved m-endoagar plates dried over night, after incubation at 37°C for 24 hrs, the colonies with a green metallic sheen were counted as presumptive members of total coliforms group **(APHA, 1981).**

Chemical analysis

All the following parameters were determined in wastewater during the treatment time (20 days).

BOD was determined according to the Standard Methods for the Examination of water and wastewater **(1985)**. COD According to Annual Book of ASTM Standard **(1976).** Total phosphorus was determined by stannous chloride method as described by standard methods for the examination of water and wastewater **(1985).** Ammonium-N, nitrate-N, nitrite-N According to **Markus *et al*., (1982).**

Nutrient removal rates were calculated by dividing the difference between the first day and next day concentrations by the first day concentration, then multiplied by 100 and expressed as percentage.

Heavy metals analysis

Analysis of heavy metals, Ni, Mn, Fe, Cu, Zn, Cr, Mo, Al, Si, V, Ti, Sr were determined using Perkin–Elmer atomic absorption spectrophotometer model 2380 before and after wastewater treatment by the method described by **(Singh *et al.* 1989).**

Statistical analysis

Data obtained in the current study were statistically analyzed using the least significant difference (LSD) at levels $p < 0.05$ and $p< 0.01$ using the program (SPSS package, version 16)**.**

3. Result and discussion

Growth rate of *Chlorella* sp. cultivated in mixed domestic-industrial wastewater

Microalgae *Chlorella* sp. showed a good growth potential in both Z-medium and wastewater, cell count of *Chlorella* sp. in both media showed rapid increase in the algal density and recorded its maximum count 27.3×10^6 unit ml^{-1} after 16 days in Z-medium, while in wastewater medium the cell count recorded 22.3×10^6 unit ml^{-1} after 20 days of incubation, there was significant difference between Z-medium and natural wastewater in the cell counting at $p <0.05$.

Chlorophyll (a) content reached the maximum value (5.65 mg l^{-1}) in Z-medium, on the other hand, in wastewater chlorophyll (a) recorded 4.55 mg l^{-1} after 20 days. The difference between Chlorophyll (a) content of *Chlorella* sp. grown in the synthetic Z-medium and natural wastewater was statistically non significant at $p < 0.01$.

Chlorophyll (b) showed significant increase when grown in both Z-medium and wastewater. After 16 days Chlorophyll (b) content reached the maximum value (2.85 mg l^{-1}) in Z-medium, and in wastewater chlorophyll (b) recorded 2.32 mg l^{-1} after 20 days. Statistically, there was no significant difference between the Chlorophyll (b) content in Z-medium and wastewater at $p < 0.01$.

Dry weight of *Chlorella* sp. showed significant increase in Z-medium and reach its maximum (1.42 gm l^{-1}) after 16 days and (1.13 gm l^{-1}) in wastewater medium after 20 days. The difference between dry weight of *Chlorella* sp. grown in the synthetic Z-medium and natural wastewater was statistically non significant at $p < 0.01$.The obtained growth data of both *Chlorella* sp. in crude wastewater showed a significant increase in microalgal density. This revealed that wastewater acted as a stimulatory agent for microalgal growth, this may be due to the presence of available organic matter, phosphorus and nitrogenous compounds which support microbial growth **(Ajayan *et al*., 2011**).

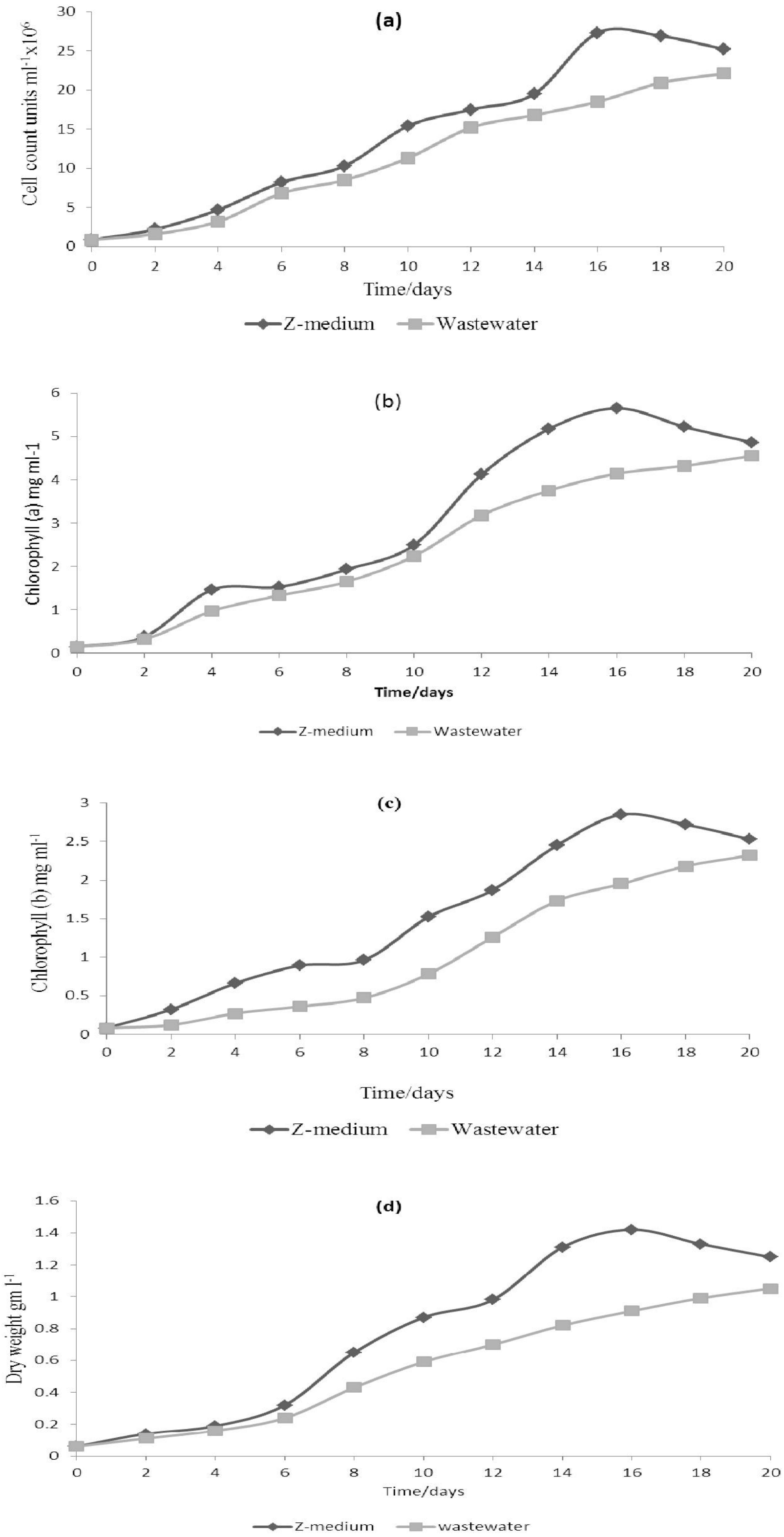

Figure (1): growth of *Chlorella sp*. in natural medium (wastewater) and synthetic medium (Z-medium) during treatment period (20 days) which represented by a) cell count (units ml^{-1}), b) chlorophyll (a) content (mg l^{-1}), c) chlorophyll (b) content (mg l^{-1}), d) dry weight (gm l^{-1}), Values are mean of three replicate.

Wastewater quality

Table (1) shows some of the physico-chemical characteristics of wastewater under experimental study, at El alalma wastewater treatment station; it has a color and un-acceptable odor. During the study period (20 days), PH value recorded 7.8 which tended to slightly alkaline. Electrical conductivity (E.C.) was estimated of 3.17 mmohs. cm^{-1}.

The total soluble salts (T.S.S.) of the wastewater sample in the present study recorded 266 mg l^{-1}. The initial chemical analysis revealed high contents of nitrogen and phosphorus as (ammonia, nitrate, and phosphate) they recorded 38, 30, and 12 mg l^{-1} respectively. Biological oxygen demand (BOD) and chemical oxygen demand (COD) contents of the wastewater sample were 316 mg l^{-1} and 627 mg l^{-1} respectively. The recorded anions were carbonate (CO_3^{--}), bicarbonate (HCO_3^-), sulphate (SO_4^{--}) and chloride (Cl^-). Chloride content of wastewater sample recorded 7.3 mg l^{-1}. sulphate content recorded 3.1 mg l^{-1}. The bicarbonate content recorded15.6 mg l^{-1}, on the other hand carbonate ions recorded 2.4 mg l^{-1}.

The detected cations in wastewater sample were restrictive to Ca^{++}, Mg^{++}, Na^+, K^+. Calcium and magnesium contents recorded an average 102.7 and 63.4 ppm, respectively. On the other hand, sodium content recorded the highest cation concentration 206.4 ppm, while potassium displayed the lowest with average of 25.2 ppm.

Table (1): Physico-chemical characteristics of domestic-industrial wastewater in El-Alalma wastewater treatment station.

Parameter	Domestic-Industrial wastewater
Physical characteristics	
Color	Dark gray
Odor	Un-acceptable
PH	7.8±0.20
E.C	3.17±0.12 mmohs. cm^{-1}
T.S.S	266 mg l^{-1}
BOD	316±5.56 mg l^{-1}
COD	627±7.0 mg l^{-1}
Ammonium	38±0.36 mg l^{-1}
Nitrate	30±0.26 mg l^{-1}
Nitrite	0.6±0.002mg l^{-1}
Phosphate	12±0.45 mg l^{-1}
Chloride	7.3mg l^{-1}
Carbonate	2.4mg l^{-1}
Bicarbonate	15.6 mg l^{-1}
Sulphate	3.1mg l^{-1}
Sodium	206.4 ppm
Potassium	25.2ppm
Calcium	102.7ppm
Magnesium	63.4ppm

pH

After the incubation period the pH value was changed to the alkaline side, pH increased gradually from 7.8 to 10.7 in *Chlorella* sp. reactor, this general tendency to the alkaline side may be due to the increased photosynthetic activity of planktonic algae, or to the chemicals nature of water **(Fathi *et al.*, 2013)**. Variation in pH can affect metabolism and growth of algae in a number of ways, including altering the equilibrium of inorganic carbon (C) species, changing availability of nutrients, and, at extremes, directly affecting cell physiology **(Mostafa, 2010).**

Table (2): Variations of pH values in wastewater during the growth of*Chlorella* sp.and in the control reactor.

Time	Wastewater (Control)	*Chlorella* sp.
0	7.8±0.20	7.8±0.20
2	7.7±0.54	8.5±0.26
4	8.1±0.25	8.8±0.17
6	8.7±0.26	9.5±0.20
8	8.9±0.65	9.95±0.13
10	9.0±0.30	10.1±0.10
12	9.1±0.26	10.4±0.36
14	8.9±0.35	10.5±0.11
16	8.7±0.71	10.7±0.17
18	8.5±0.40	10.6±0.26
20	8.1±0.36	10.2±0.21

Data are expressed as mean of three replicate ± SD (standard deviation).

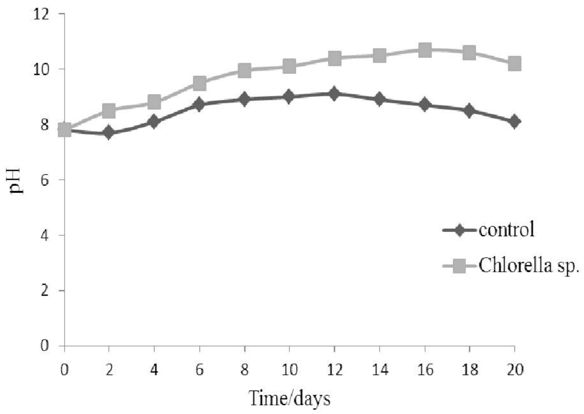

Figure (2): Variations of pH values in wastewater during the experimental period (20 days) in control (wastewater) reactor and *Chlorella* sp. reactor.

Coliform bacteria removal

The estimation of total coliforms in both reactors *Chlorella* sp. and in wastewater during the treatment period showed a significant drop in the bacterial count and accounted for 99.9% removal efficiency in *Chlorella* sp. after 8 days, but removal efficiency 99.9% in the control (wastewater) reactor was achieved after 18 days. These results was in agreement with **Marchello *et al.*, (2015)** who showed that the reduction of colony forming units for both total coliforms and for *E. coli* occurred abruptly in the first 2 days, no longer detected after the 18th day and the 11th day for total coliform during aerated and non aerated treatment respectively, and **Hassan, (2001)** who reported a remarkable decrease in the number of coliform 99.99 and 99.8 % during wastewater treatment by *Phormidium sp.* and *Aphanocapsa sp.* respectively. **Amengual-Morro *et al.*, (2012)** recorded that the microlagae photosynthetic activity in sewage treatment effluents overcomes bacteria respiration during daytime, leading to an imbalance in pH between day and night, this leads to the inactivation of coliform and other bacteria forms.

Table (3): Total coliform bacteria (cfu ml^{-1}) and its removal efficiency in wastewater after 20 days treatment in control (wastewater) reactor and *Chlorella* sp. reactor.

Time in days	Control (wastewater)		*Chlorella* sp.	
	Cfu ml^{-1}	% of removal	Cfuml^{-1}	% of Removal
0	24x10^{6}±2.64x10^{6}	0	24x10^{6}±2.64x10^{6}	0
2	91x10^{5}±2.21x10^{5}	62.08	32x10^{5}±2.53x10^{5}	86.6
4	90x10^{5}±3.53x10^{5}	62.5	36x10^{4}±1.85x10^{4}	98.5
6	35x10^{4}±2.56x10^{4}	98.54	4x10^{4}± 0.22x10^{4}	99.8
8	18x 10^{4}± 9.0x10^{3}	99.25	10^{3}± 52.63	99.99
10	8x 10^{4}±1.73x10^{3}	99.66	ND	_
12	19x 10^{3}± 3.0x10^{2}	99.92	ND	_
14	14x10^{3}±1.25x10^{2}	99.94	ND	_
16	10^{4}± 0.56x10^{2}	99.95	ND	_
18	2x10^{3}± 87.43	99.99	ND	_
20	ND	_	ND	_

Data are expressed as mean of three replicate ± SD (standard deviation).

Table (4): Ammonium-Nitrogen concentrations (mg l^{-1}) and its removal efficiency in wastewater after 20 days treatment in the control (wastewater) reactor and *Chlorella* sp. reactor.

Time in days	Wastewater(control)		*Chlorella* sp.	
	Conc. mgl^{-1}	% of removal	Conc. mgl^{-1}	% of removal
0	38±0.36	0	38±0.36	0
2	35±0.43	7.9	27.5±0.45	27.6
4	30.2±0.26	20.5	22±0.55	42.1
6	27.8±0.30	25	18.8±0.36	50.5
8	28.5±0.56	29.5	19±0.43	50
10	25±1.04	34.2	9.5±0.69	75
12	23.3±0.72	38.7	5.8±0.25	84.73
14	23.8±0.55	37.4	4.2±0.15	88.94
16	21.7±0.26	42.9	2.5±0.40	93.4
18	24.5±0.36	35.5	1.1±0.23	97.1
20	20.6±0.72	45.8	0.4±0.15	98.9

Data are expressed as mean of three replicate± SD (standard deviation).

Removal of ammonium-Nitrogen

Chlorella sp. proved high ability to remove NH_4^+-N from wastewater, the percentage of ammonia removal was 98.9 % after 20 days of incubation, On the other hand; only 45.8 % removal efficiency was recorded in the control reactor. The NH_4^+-N removal efficiency achieved in this study was higher, compared to that of other studies; **Abinandan *et al.*, (2013)** reported that removal percentage of Ammonia-Nitrogen after the end of batch process is 58.8% **(Wang *et al.*, 2013)** who reported that the removal rate of $NH4^+$-N was higher than 83% in infflunet wastewater, especially under 75%- 50 % 25% wastewater conditions. More than 90% $NH4^+$-N in the wastewater sample had been absorbed by the microalgae **(Sharma and Khan, 2013).**

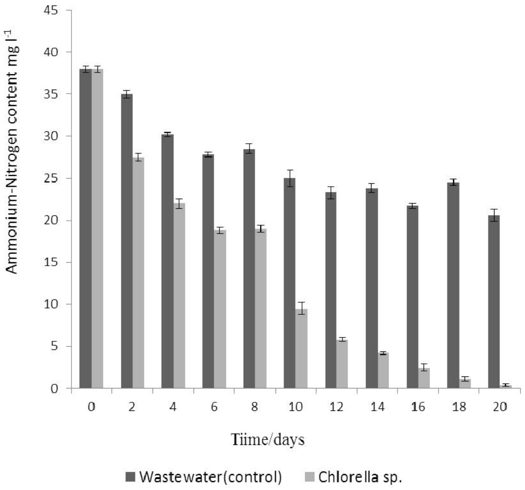

Figure (3): Ammonium-Nitrogen concentrations (mg l^{-1}) in wastewater treated in the control (wastewater) and *chlorella* sp. reactors during the experiment period (20 days).

The result observed in the present study was in harmony with **Li *et al.* (2011)** who evaluate the feasibility of growing *Chlorella* sp. on centrate wastewater and the results showed that the algae removed ammonia as high as 93.9%, and **Woertz *et al.* (2009)** treated municipal wastewater in semi-continuous indoor cultures with 2-4 day hydraulic residence times (HRTs). Over 99% removal of ammonium was achieved in this experiment. Similarly, *Chlamydomonas* sp. has been reported to remove 100% of NH4+ when grown in raw industrial wastewater containing 38.4 mg L-1 $NH4^+$-N **(Wu *et al.*, 2012)**.

Removal of Nitrate- Nitrogen (NO_3^--N):

Data in table(5) showed that the maximum removal efficiency of NO_3^--N achieved by *Chlorella* sp. was 87.3 % and 41.7 % in the control reactors at the end of incubation period (20 days).This result was in agreement with **Sharma *et al.*, (2015)** who indicated that *Chlorella pyrenoidosa* achieved removal of around 76 % of the concentration of nitrate in the biogas wastewater in the 15 days of inoculation. Similar trend was recorded by findings of **Sengar *et al.*, (2011)** are supporting the above trend who also noted 91 % reduction in NO_3^--N using mixed algal population. Nitrate was reduced very appreciably by 81% **(Sivasubramanian *et al.*, 2012).**

Microalgae prefer to assimilate nitrogen in the form of ammonia because it is a passive way of assimilation and energetically less expensive than uptake of nitrate **(Sharma and Khan, 2013).**

The significant N reduction observed in the present research may be attributed to aeration, this result was recorded in other investigations and can be related to the sum of processes occurring simultaneously while bubbling, *e.g.*, nitrification, consumption of $NH4^+$ by microalgae and elimination of NH3 to the atmosphere **(Zhang *et al.*, 2011; Ray *et al.*, 2012; Marchello *et al.*, 2015).**

Table (5): Nitrate-Nitrogen concentrations (mg l^{-1}) and its removal efficiency in wastewater after 20 days treatment in the reactors; control (wastewater) reactor and *Chlorella* sp.

Time in days	Control (wastewater)		*Chlorella* sp.	
	Conc. mgl^{-1}	% of removal	Conc. mgl^{-1}	% of removal
0	30±0.26	0	30±0.26	0
2	27.3±0.22	0	22.5±0.41	25
4	26.4±0.31	8.6	18.6±0.43	38
6	26.7±0.41	15	15.2±0.33	49.3
8	22.6±0.10	28	13.5±0.30	55
10	20±0.23	33.4	11.8±0.36	60.6
12	19.7±0.51	34.3	8.3±0.40	72.3
14	17.1±0.40	45	8±0.51	73.4
16	18.3±0.36	39	6.8±0.22	77.3
18	17.8±0.40	42.6	5.2±0.32	82.7
20	17.5±0.36	41.7	3.8±0.16	87.3

Data are expressed as mean of three replicate ± SD (standard deviation)

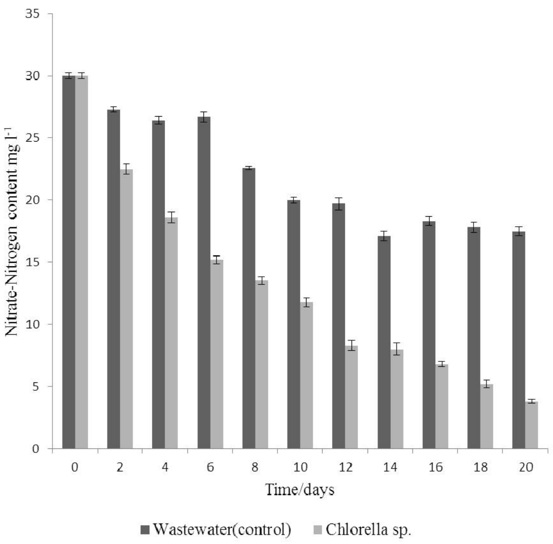

Figure (4): Nitrate-Nitrogen concentrations (mg l^{-1}) in wastewater treated in the control (wastewater) and *Chlorella* sp. reactors during the experimental period (20 days). Values are the mean of three replicates.

Phosphorus removal

Treatment of the mixed domestic-industrial wastewater in the current study by microalgae *Chlorella* sp. induced significant reduction in phosphorus concentration with increasing treatment time. in *Chlorella* reactor, 90 % phosphorus removal rate was recorded at the end of the treatment period, On the other hand, 27.5 % removal rate was recorded in the control reactor after 20 days of incubation period. These results was in harmony with **(Velan and Saravanane, 2013)** obtained a maximum removal efficiency of 87% and **Cho *et al.*, (2011)** who conducted an experiment in municipal wastewater and the maximum removal efficiency obtained was about 86% and **Li *et al.*, (2011)** reported that *Chlorella* sp. removed total P, as high as 80.9% from centrate wastewater. Phosphorus removal rate was higher than that of **(Wu *et al.*, 2012**) who reported that *Chlamydomonas* sp. removed 33% of PO_4^{3-}. when grown in raw industrial wastewater containing 44.7 mg l^{-1} PO_4^{3-}Phosphorus can be eliminated through both biotic phosphorous assimilation into the microalgal biomass and a biotic phosphorous precipitation **(Godos *et al.*, 2009). Su *et al.*, (2011)** reported on abiotic Phosphorus removal which took place mainly in the form of orthophosphate precipitation at high pH (9-11).

Table (6): Phosphorus concentrations (mg l^{-1}) and its removal efficiency in wastewater after 20 days treatment in control (wastewater) and *Chlorella* sp. reactors.

Time in days	Control (wastewater)		*Chlorella* sp.	
	Conc. mgl^{-1}	% of removal	Conc. mgl^{-1}	% of removal
0	12±0.45	0	12±0.45	0
2	11.8±0.26	1.66	9.6±0.15	20
4	11.2±0.10	6.7	8.4±0.10	30
6	11.5±0.43	4.2	8±0z36	33.3
8	10.9±0.20	9.2	7.1±0.30	40.8
10	10.5±0.24	12.5	5.7±0.28	52.5
12	9.53±0.15	20.58	4.8±0.26	60
14	9.2±0.18	23.3	4.2±0.32	65
16	10.3±0.30	14.2	3.1±0.15	74.2
18	9.7±0.42	19.2	1.8±0.22	85
20	8.7±0.15	27.5	1.2±0.27	90

Data are expressed as mean of three replicates ± SD (standard deviation).

Biological Oxygen Demand (BOD) removal

Chlorella sp. was efficient in BOD removal as the *Chlorella* sp. reactor recorded the maximum reduction 90.8% after 20 days of incubation period, while 41.5% removal efficiency was achieved in the control (wastewater) reactor.

This result is in harmony with other studies such as **Sharma and Khan *et al.*, (2013)** who recorded that *Chlorella minutissima* removed about 95% BOD content from the wastewater and **kumar and Goyal, (2010)** who found that the reduction in biochemical oxygen demand was 79% during treatment of domestic wastewater using *Chlorella* sp. **Sivasubramanian *et al.*, (2012)** recorded that *Chlorella conglomerate* and *Chlorococcum humicola*were able to reduce BOD by 77% and 86.1% respectively. **El-Bestawy (2008)** reported high efficiencies of *Anabaena variabilis* and *Anabaena oryzae* as suspended growth application toward the removal of BOD5 from mixed domestic–industrial wastewater in a relatively short duration. BOD5 recorded 89.29 as maximum RE(s) achieved by *Anabaena variabilis* and *Anabaena oryzae*, respectively.

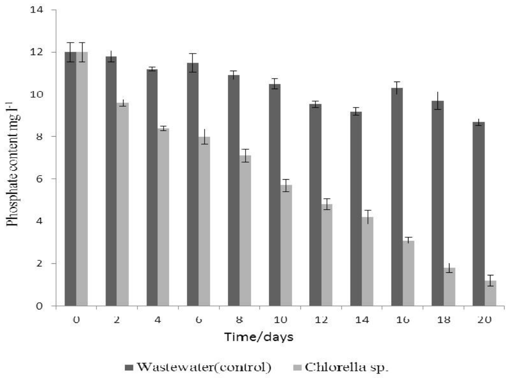

Figure (5): Phosphorus concentrations (mg l^{-1}) in wastewater treated in the control (wastewater) and *Chlorella* sp. reactor during the experiment period (20 days).

Table (7): Biological Oxygen Demand (BOD) concentrations (mg l^{-1}) and its removal efficiency in wastewater after 20 days treatment in control (wastewater) and in *Chlorella* sp. reactors.

Time in days	Control (wastewater)		*Chlorella* sp.	
	Conc. mgl^{-1}	% of removal	Conc. mgl^{-1}	% of removal
0	316±5.56	0	316±5.56	0
2	285±4.58	9.8	210±4.33	33.5
4	260±7.55	17.7	204±6.74	35.4
6	255±4.22	19.3	165.5±5.03	47.6
8	235±3.0	25.6	73±4.58	76.9
10	212±5.42	32.9	59±2.00	81.3
12	188±4.0	40.5	56±1.45	82.3
14	205±3.60	35.12	62±3.52	80.4
16	197±4.58	37.7	44.2±3.11	86.01
18	176±6.812	44.3	41±4.00	87.02
20	185±5.34	41.5	29±1.05	90.8

Data are expressed as mean of three replicates ± SD (standard deviation).

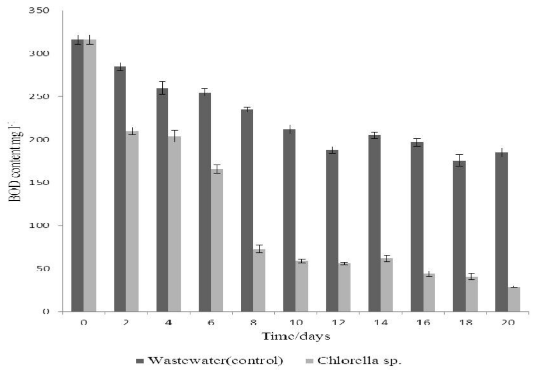

Figure (6): Variations in the levels of Biochemical oxygen demand (BOD) (mg l^{-1}) in wastewater treated in control (wastewater) reactor and *Chlorella* sp. reactors during the experiment period (20 days).

Chemical Oxygen Demand (COD) removal

In the present study, COD estimated 627 mg l^{-1}, the high amount of COD may be attributed to high concentration of xenobiotic compounds, which remain unaffected by microflora **(Garg and Tripathi, 2013).** The results showed that the maximum removal efficiency of COD achieved by *Chlorella* sp. was 80.1% while, in the wastewater reactor COD reduced by 40.4 % at the end of incubation period (20 days).This result was in agreement with that of previous studies such as **Sivasubramanian *et al.*, (2012)** who reported that *Chlorella conglomerate* and *Chlorococcum humicola* were able to reduce COD by 77.7 % and 85.9 % respectively and **Sharma and Khan, (2013).** *Chlorella minutissima* was found to remove about 90% COD from the wastewater**.** This result is quite similar to the study done by **Mamun *et al.*, (2012),** reported that the initial concentration of COD was 90 mg/L, while the final concentration was 20 mg/L with The maximum value of 77.8% percent removal of COD occurred at day 14th day. **kumar and Goyal, (2010),** recorded reduction in chemical oxygen demand about 93% during treatment of domestic wastewater using *Chlorella* sp. **(Mata *et al*., 2012),** observed that with the culture aeration higher values of algal biomass growth are obtained due to increased photosynthetic activity of microalgae. Therefore, more oxygen is generated, helping to reduce the COD and making the treatment more effective.

Table (8): Chemical Oxygen Demand (COD) concentrations (mg l $^{-1}$) and its removal efficiency in wastewater after 20 days treatment in; control (wastewater) *Chlorella* sp. reactors.

Time in days	**Control (wastewater)**		***Chlorella* sp.**	
	Conc. mgl^{-1}	**% of removal**	**Conc. mgl^{-1}**	**% of removal**
0	627±7.00	0	627±7.00	0
2	591±4.51	5.7	498±6.050	20.6
4	520±7.35	17.06	387±9.50	38.3
6	460±6.08	26.6	340±5.52	45.8
8	467±7.15	25.5	308±7.66	50.9
10	395±6.31	37	262±4.00	58.2
12	439±9.00	29.9	215±6.50	65.7
14	406±8.87	35.2	224±8.41	64.3
16	396±5.58	36.8	202±5.56	67.8
18	385±9.60	38.6	158±6.55	74.8
20	374±5.00	40.4	125±4.23	80.1

Data are expressed as mean of three replicate ± SD (standard deviation).

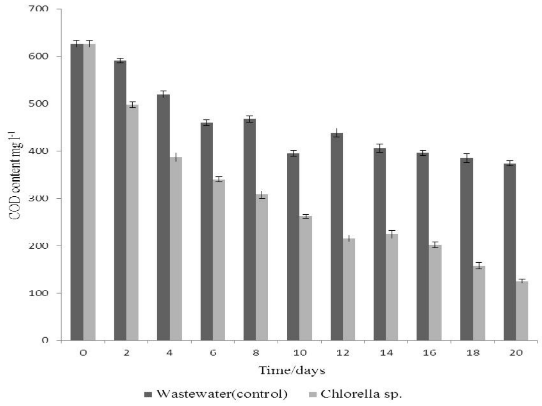

Figure (7): variations in the levels of chemical oxygen demand (COD) (mg l^{-1}) in wastewater treated in control (wastewater) and *Chlorella* sp. reactors during the experiment period (20 days).

Heavy metal removal by microalgae *Chlorella* sp.

In the present study the removal efficiency of nickel, manganese, iron, copper, zinc, chromium, molybdenum, aluminum, silicon, vanadium, titanium, strontium was detected. *Chlorella* sp. recorded heavy metal removal efficiency 99.5 % for nickel, 73.2 % for manganese, 92.2 % for iron, 54.5 % for copper, 51.4 % for zinc, 56.3 % for chromium, 99.7 % for molybdenum, 98.8 % for aluminum, 48.5 % for silicon, 100 % for vanadium, 100 % for titanium, 41.9 % for strontium.

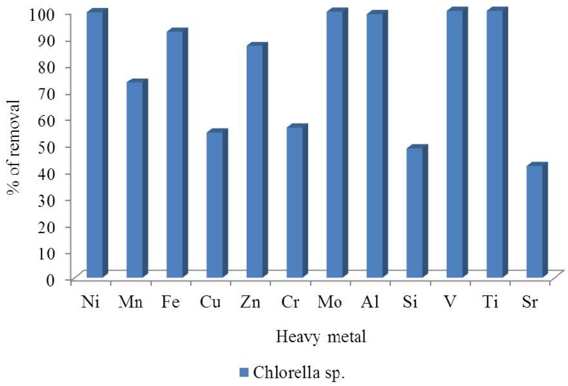

Figure (18): Heavy metals removal from wastewater after the treatment period (20 days) in *Chlorella* sp. and *Chroococcus* sp. reactors.

(Fathi *et al* 2013) indicted that cultivation of green alga *Chlorella vulgaris* on wastewater has a positive effect on removal the major inorganic elements form the wastewater.60–70% removal of Zn^{2+} was observed from culture medium containing 5-20 mg L^{-1} Zn^{2+} by *Chlorella* sp. in the stabilization pond water **(kumar and Goyal, 2010). Ajayan *et al*., (2011)** reported that *Scenedesmus bijuga* and *Oscillatoria quadripunctulata* showed heavy metal removal capacity 37-50 % for copper, 20-33% for cobalt, 35-100% for lead and 32-100% in case of zinc from the sewage and petrochemical industry effluent. Mn, and Zn were found to be removed very efficiently from all the wastewaters with different concentrations, with removal rates ranging from 56.5% to 100% **(Wang *et al*., 2010). Dwivedi, (2012)** described two steps involved in the assimilation of heavy metals. First, the metals are adsorbed over the cell very quickly called physical adsorption. Next, these metals are assimilated slowly into the cytoplasm in a process named chemisorptions.

Conclusion

Our results showed that domestic-industrial wastewater could promote good algal growth of *Chlorella* sp. to a similar extent as observed for the Z-medium, at the same time *Chlorella* sp. exhibited appreciable removal capacities of nutrients (ammonium-nitrogen, nitrate-nitrogen, phosphorus), BOD, COD, eliminating of coliform bacteria and heavy metals. Therefore it is clear that the treatment approach using *Chlorella* sp. offers a low-cost, efficient and environmentally friendly technology for the treatment of mixed of domestic-industrial wastewater.

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