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Reducing the Pollutants from Municipal Wastewater by Chlorella Vulgaris Microalgae

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Abstract

In the present work, the pollutants of the municipal wastewater are reduced using *Chlorella vulgaris* microalgae. The pollutants that were treated are: Total organic carbon (TOC), Chemical oxygen demand (COD), Nitrate (NO_3), and Phosphate (PO_4). Firstly, the treatment was achieved at atmospheric conditions (Temperature = 25^{o}C), pH 7 with time (1 – 48 h). To study the effect of other microorganisms on the reduction of pollutants, sterilized wastewater and unsterilized wastewater were used for two types of packing (cylindrical plastic and cubic polystyrene) as well as algae's broth (without packing), where the microalgae are grown on the packing then transported to the wastewater for treatment. The results showed that the other microorganism in unsterilized wastewater can slightly contribute in the treatment. Packing of cylindrical plastic is more effective than the cubic polystyrene, and microalgae's broth gives better results than the two types of packing. The treatment in the first hours was performed quickly while in the last hours, it was very slow. Then, the following parameters in the range of (temperature: 20 – 35^o) pH (5 – 8), volume ratio of wastewater to microalgae's broth (1 – 2.5) were studied for sterilized wastewater and constant treatment time equal to 48 h. The results showed that the maximum reduction of pollutants are: TOC = 92.3%, NO_3 = 65.2%, PO_4 = 93.2% at T = 35^{o}C, pH 8, and (wastewater/algae broth) ratio = 1, and COD = 85.6% at T = 30^{o}C, pH 7, and (wastewater/ algae broth) ratio = 1. The temperature and pH have little effect on the reduction of pollutants compared with the wastewater/algae broth ratio. The adsorption isotherm for pollutant was also studied for three types of isotherm; linear, Freundlich, and Langmuir. The results showed that the treated pollutants are the Langmiur adsorption isotherm.

Keywords: *Biofiltration, microalgae, treatment, Wastewater.*

1. Introduction

The municipal wastewater have many pollutants such as nitrate, phosphorus, CO_2, TOC, etc of the organic and inorganic pollutants that cause increasing in the BOD, COD which harm the environment dramatically [1, 2]. So it is necessary to treat the pollutants in wastewater before discharge to the river. There are many methods to treat the pollutants in wastewater named physical treatment such as screening, sedimentation, precipitation, aeration, filtration, …etc, chemical treatment such as chlorination, ozonation, coagulation, ion exchange, …etc, and biological treatment such as activated sludge treatment methods, trickling filtration, oxidation ponds, lagoons, anaerobic digestion, septic tanks, etc [3]. Present of pollutant in the water bodies on one hand aggravates the phenomena such as algal flowers and on the other hand influents the ecological balance of water and subsequently decays the water characteristic [4]. Microalgae are widely used as a multi-biotreatment process to reduce nitrogen and phosphorus from wastewater since they require nitrogen, phosphorus, CO2, and light for their autotrophic metabolic growth [5, 6]. The process of microalgae is an effective and little technology process which offers inveterate cost savings and provides a more suitable method of water treatment for improving countries [7]. One of the main features of algal processes over classical treatment is the ability to recycle the nutrients forming rich value products, such fertilizers, medicinal products, food additives and biofuels [8, 9, 10]. *Chlorrela vulgaris* is a unicellular microalgae present individually or in flocks that consume carbon dioxide, nitrogen, and phosphorous and release oxygen [11]. The main objectives of the present work are to reduce the pollutants from wastewater at optimum operating conditions.

2. Experimental Procedure

2.1. Materials

- Wastewater: It is taken from municipal of Baghdad-Aljaderia.
- Chlorrela Vulgaris microalgae: It is obtained from Ministry of science and technology/ Office of Research and technology of environment and water.
- Packing: Two type of packing are used; Cylindrical plastic (polyethylene-glycol, PVC) with dimensions: length = 1 cm, diameter = 0.8 cm, thickness = 0.1 cm and has specific surface area = 261 m^2/m^3.
  Cubic polystyrene: Its dimensions are 1×1×1 cm and has specific surface area = 180 m^2/m^3.
- BG-11 Media: It is composed of (g/l): $NaNO_3$ 150, K_2HPO_4 4.0, $MgSO_4.7H_2O$ 7.5, $CaCl_2.2H_2O$ 3.6. Citric acid 0.6, Ferric citrate 0.6, EDTA-Na 0.1, Na_2CO_3 2.0, and Micronutrient solution 0.532. The source of this media is HIMEDIA, India. It is support the growth of microalgae.

2.2. Experimental Procedure

Cultivation media is prepared by suspension 1.627 g of BG-11 per liter of distilled water then autoclaved at 121ºC for 20 min. After cooling the prepared media solution to the room temperature, add 250 ml of solution into flasks each capacity 500 ml. The isolated microalgae are cultured in each flask, the flasks are stoppered by cotton, aerated by 0.5 lit/min sterilized filtered air (through milipore filter 0.25µm) and exposure to neon light intensity of 2500-2600 lux measured by (Photometer milkwaukee, China). The temperature is maintained at 25ºC by kept the flasks in incubator [12]. The growth period of microalgae is 7 days. The broth solution now is ready to use in the treatment.

Wastewater is filtered by filter paper and sterilized at 121ºC for 20 min to ensure there is no microorganisms are present and the microalgae will be alone that used in the treatment. The columns with capacity of 500 ml are used for treatment where 250 ml of wastewater added to the columns and 250 ml of microalgae broth are added to the wastewater where the ratio of wastewater/algae broth becomes 1. The pH of the contents is adjusted to 7. The columns are stoppered from one end with teflon and the other end with cotton. The columns are aerated by 0.5 lit/min filtered air and exposure to neon light intensity of 2500-2600 lux. The temperature is maintained at 25ºC by kept the columns in incubator. The samples (5ml) are taken with time for analyses and measure the pollutants.

Figure 1 shows the process flow diagram of the wastewater treatment by microalgae.

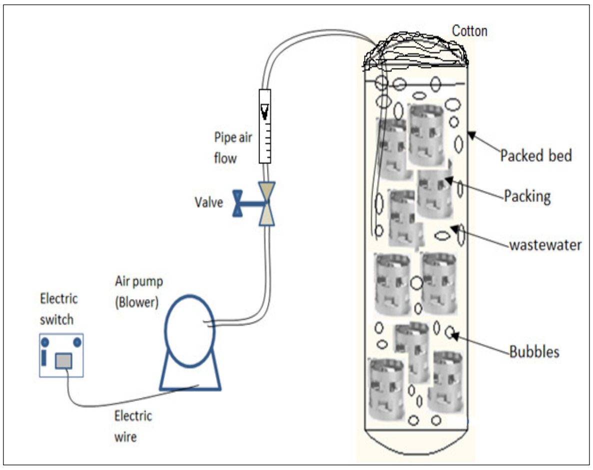

Fig. 1. Process flow diagram of the wastewater treatment by microalgae.

Table 1 shows the method of measurement of the pollutants.

Table 1,
The method of measurement of the pollutants

Pollutants (mg/l)	Measurement method
TOC	UV light and a digesting reagent, sodium persulphate
COD	UV-VIS Spectroscopy
NO_3	UV-Spectrophotometer
PO_4	UV-Spectrophotometer

3. Results and Discussion

3.1. Time Effect

Figures 1 – 4 show the concentration of the pollutants TOC, COD, NO_3, and PO_4 with time at temperature of 25°C, pH 7, wastewater/(Algae broth) volume ratio = 1, and sterilized wastewater. The wastewater is sterilized in autoclave at 121°C for 20 min to eliminate from any microorganism can contribute in treatment and microalgae will evaluated individually as remediation tool.

It can be seen from figures 1 – 4 that the pollutants are decreased rapidly in the first hours then decreased slowly with progress of time. The experimental operation time is stopped at 48 h because the decreasing of pollutants becomes very slowly.

Figure 5 shows the removal percent of pollutants with time at temperature of 25°C, pH 7, wastewater/(Algae broth) volume ratio = 1, and sterilized wastewater. It can be seen that the maximum removal percent of TOC = 87%, PO_4 = 86%, COD = 78%, and NO_3 = 50.7% after 48 h.

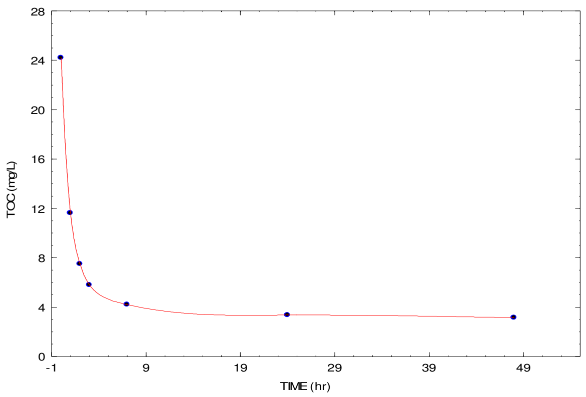

Fig. 1. TOC Pollutant concentration with time at T= 25°C, pH 7, wastewater/(Algae broth) ratio = 1, and sterilized wastewater.

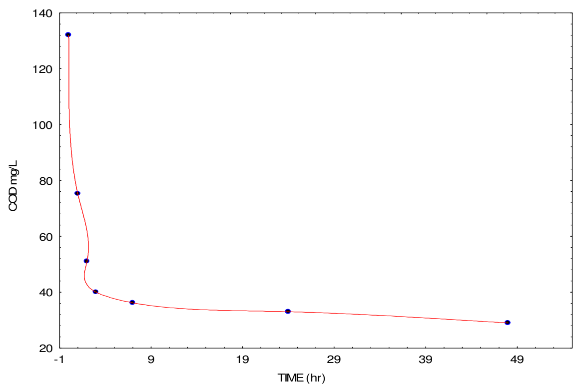

Fig. 2. COD Pollutant concentration with time at T= 25°C, pH 7, wastewater/(Algae broth) ratio = 1, and sterilized wastewater.

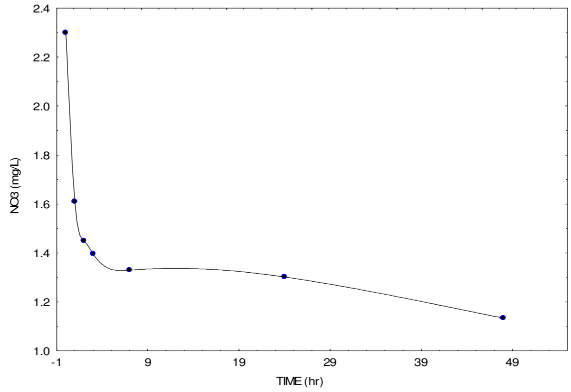

Fig. 3. NO_3 Pollutant concentration with time at T = 25°C, pH 7, wastewater/(Algae broth) ratio = 1, and sterilized wastewater.

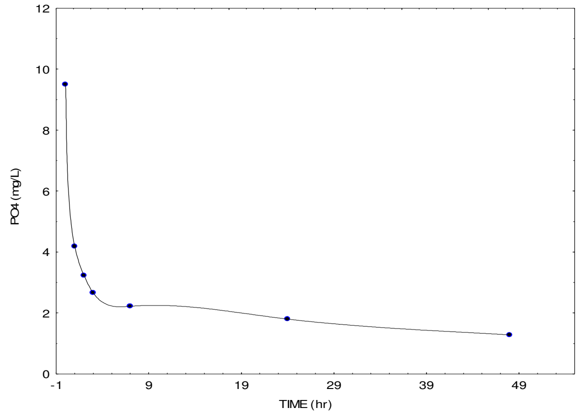

Fig. 4. PO_4 Pollutant concentration with time at T = 25°C, pH 7, wastewater/(Algae broth) ratio = 1, and sterilized wastewater.

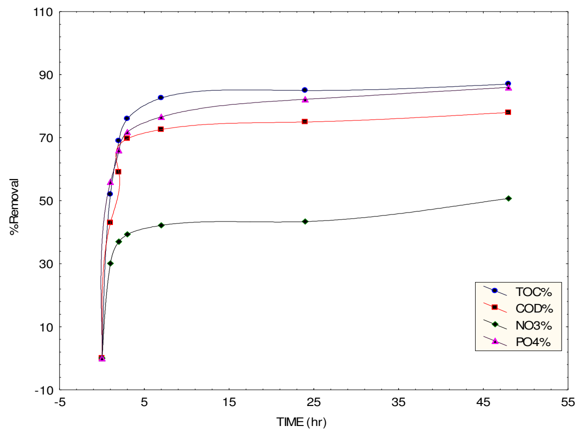

Fig. 5. Removal per cent of Pollutants with time at T = 25°C, pH 7, wastewater/(Algae broth) ratio = 1, and sterilized wastewater.

3.2. Sterilization Effect

To show the effect of microorganisms on the concentration and consumption of pollutants, the wastewater is taken directly (without sterilization) for treatment.

Figures 6 – 13 show the difference between the sterilized and unsterilized wastewater on the concentration level and removal percent for each pollutants. It can be seen that the efficiency of treatment in case of unsterilized wastewater is better than sterilized wastewater but the difference is little (not more than 5%). That means the effect of other microorganisms on the consumption of pollutant is low compare with microalgae.

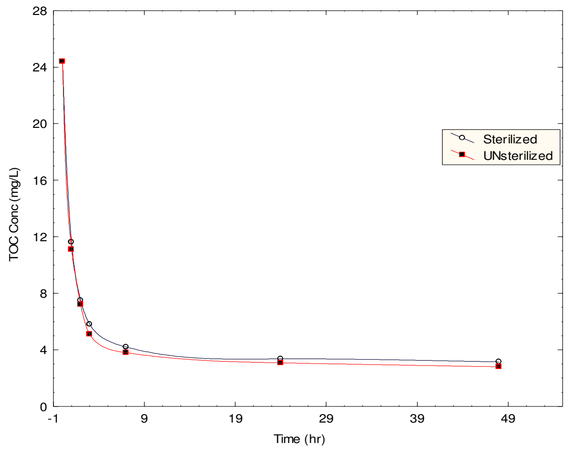

Fig. 6. Effect of sterilization on the concentration level of TOC pollutant with time at T=25°C, pH 7, wastewater/(Algae broth) ratio =1.

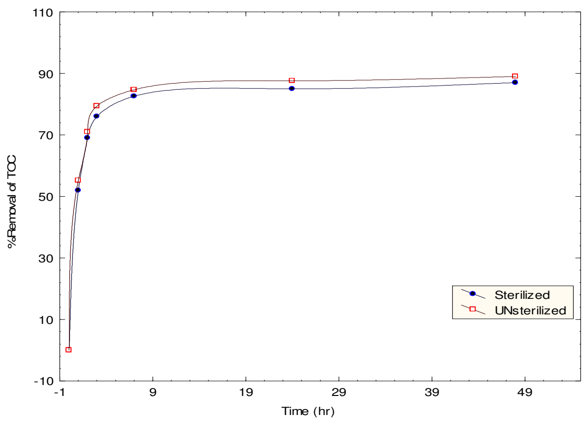

Fig. 7. Effect of sterilization on Removal percent of TOC pollutant with time at T = 25°C, pH 7, wastewater/ (Algae broth) ratio = 1.

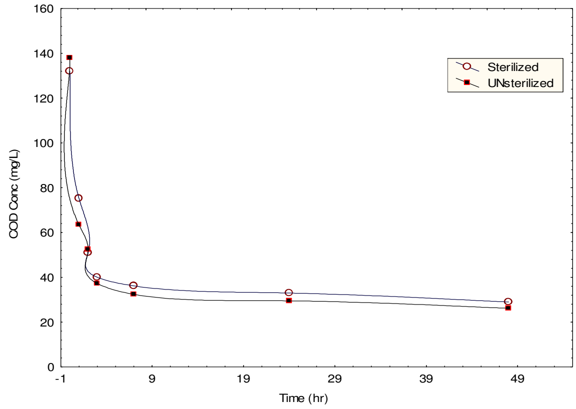

Fig. 8. Effect of sterilization on the concentration level of COD pollutant with time at T=25°C, pH 7, wastewater/(Algae broth) ratio =1.

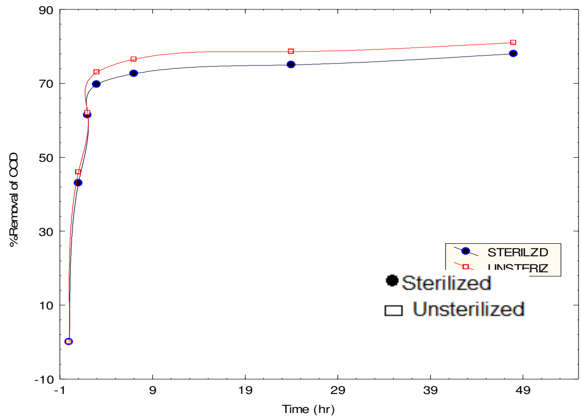

Fig. 9. Effect of sterilization on Removal percent of COD pollutant with time at T = 25°C, pH 7, wastewater/(Algae broth) ratio = 1.

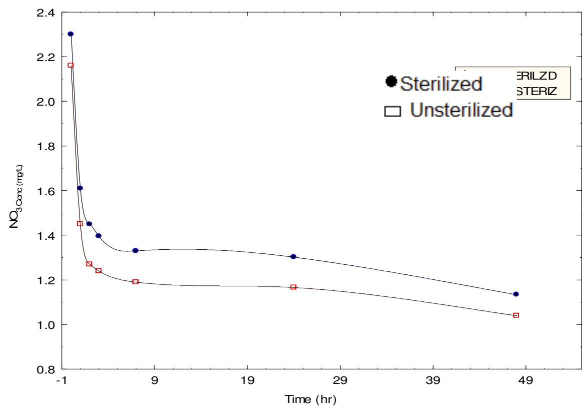

Fig. 10. Effect of sterilization on the concentration level of NO3 pollutant with time at T=25°C, pH 7, wastewater/ (Algae broth) ratio =1.

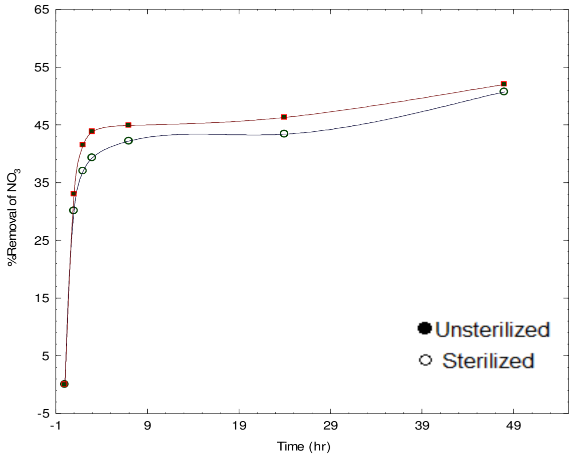

Fig. 11. Effect of sterilization on Removal percent of NO_3 pollutant with time at T = 25°C, pH 7, wastewater/ (Algae broth) ratio = 1.

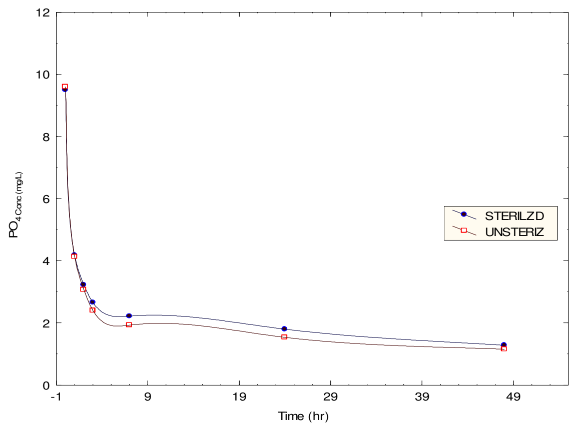

Fig. 12. Effect of sterilization on the concentration level of PO_4 pollutant with time at T=25°C, pH 7, wastewater/ (Algae broth) ratio =1.

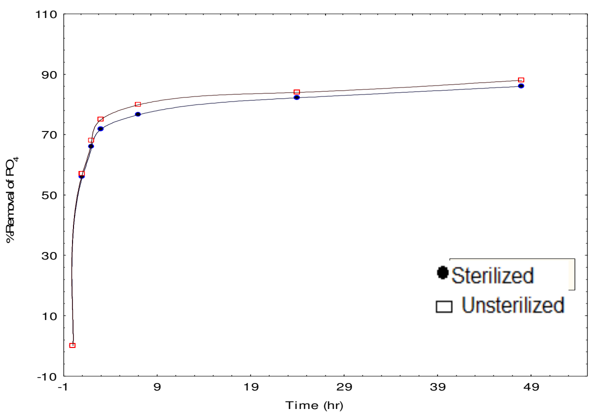

Fig. 13. Effect of sterilization Removal percent of PO_4 pollutant with time at T=25°C, pH 7, wastewater/ (Algae broth) ratio = 1.

3.3. Effect of Packing Type

The microalgae are grown on the packing and used in the treatment of pollutants. Two types of packing are used: plastic cylinder and polypropylene cubic. The amount of wastewater is still 250 ml are added to the column of treatment then add the packing where the packing is filled the entire column and the wastewater fills the porosity of the column. Figures 14 – 21 show the effect of the packing type on the concentration of pollutants and their removal percent with time. It is clear that the plastic cylinder has more efficiency on the removal of pollutants than the polystyrene cubic. The reason is the microalgae has more density on the plastic and the plastic cylinder is hollow while the polystyrene cubic is block body (without hollow), so the microalgae will grow on the outer and inner surface of plastic cylinder. Also it can be seen that from figures 14–21 the broth of algae has more efficiency than the two packing. In case of broth of microalgae, the amount of microalgae can be controlled more flexible than the packing.

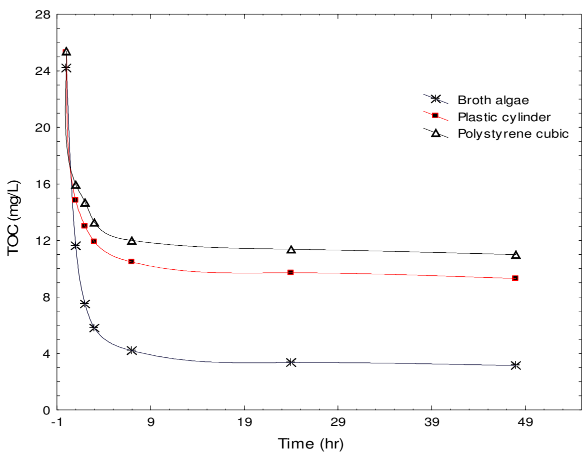

Fig. 14. Effect of Packing type on the concentration level of TOC pollutant with time at T=25°C, pH 7, and sterilized wastewater.

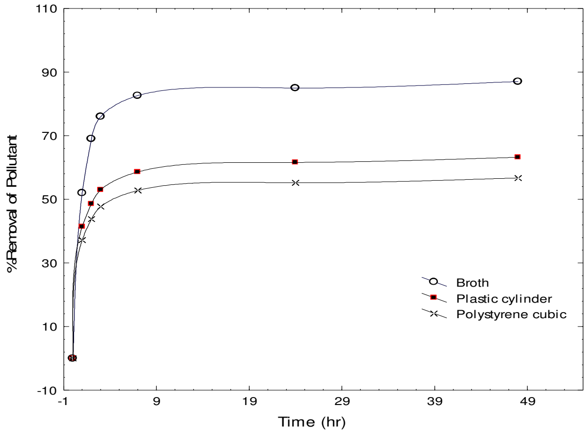

Fig. 15. Effect of Packing type on the Removal percent of TOC pollutant with time at T=25°C, pH 7, and sterilized wastewater.

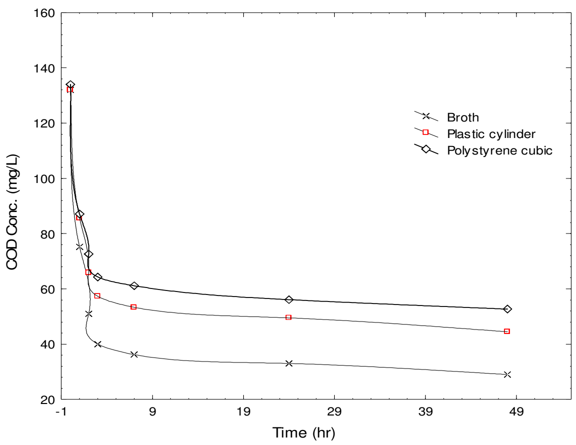

Fig. 16. Effect of Packing type on the concentration level of COD pollutant with time at T=25°C, pH 7, and sterilized wastewater.

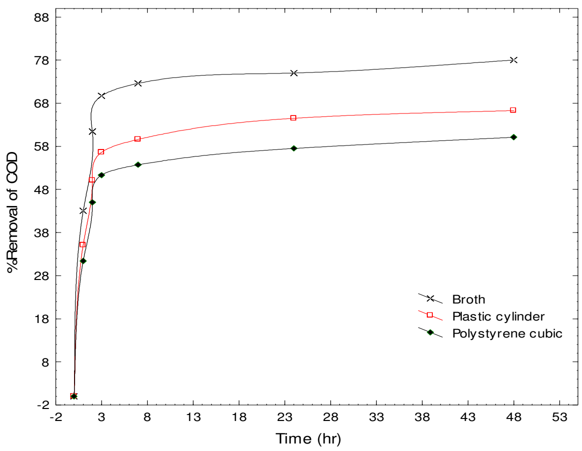

Fig. 17. Effect of Packing type on the Removal percent of COD pollutant with time at T=25°C, pH7, and sterilized wastewater.

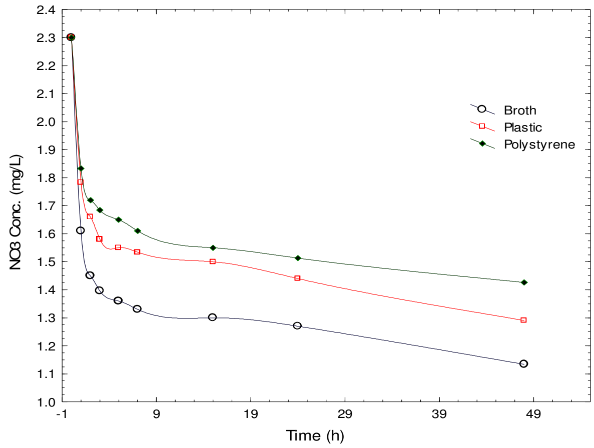

Fig. 18. Effect of Packing type on the concentration level of NO_3 pollutant with time at T=25°C, pH 7, and sterilized wastewater.

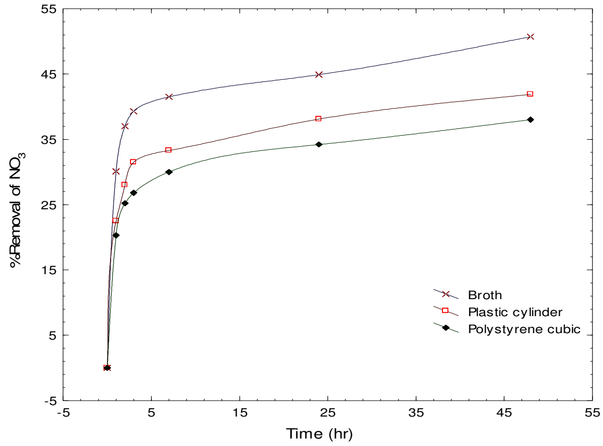

Fig. 19. Effect of Packing type on the Removal per cent of NO_3 pollutant with time at T=25°C, pH 7, and sterilized wastewater.

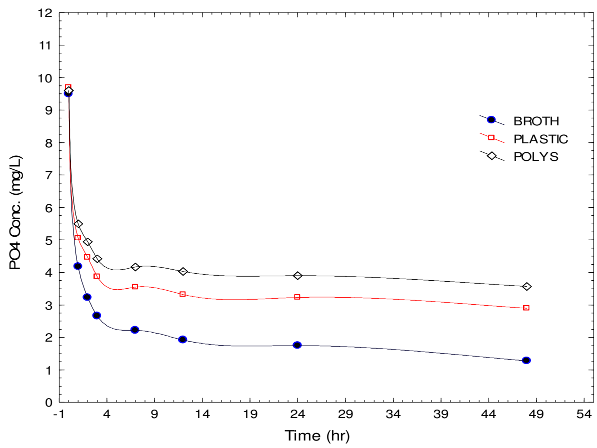

Fig. 20. Effect of Packing type on the concentration level of PO_4 pollutant with time at T=25°C, pH 7, and sterilized wastewater.

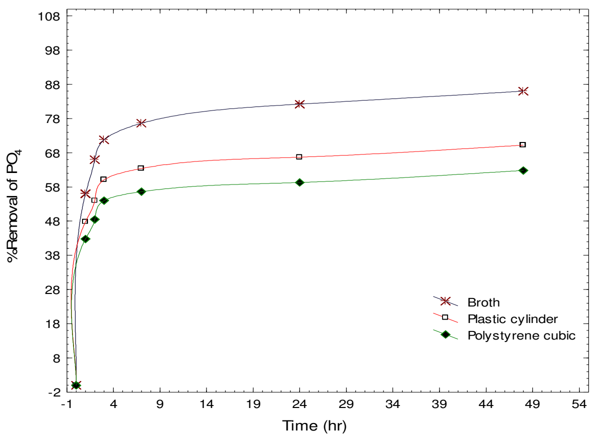

Fig. 21. Effect of Packing type on the Removal percent of PO_4 pollutant with time at T=25°C, pH7, wastewater/ (Algae packing) ratio =1, and sterilized wastewater.

3.4. Other Parameters Effect

The other parameters which are studied in reduction of pollutants are:
Temperature: 20– 35°C, pH: 5– 8, and Wastewater/ (Algae broth) volume ratio (1.0–2.5).

Design of experiments are achieved by Taguchi method in MiniTab software statistics. Table 2 shows the number of the experiments with their parameters.

According to the results of experiments in the figures (1 – 21) the microalgae broth, time of treatment (48 hr) and sterilized wastewater will used in the next experiments. Here the sterilized wastewater is dependent in this study although the unsterilized is more effective because in the next section will study the adsorption isotherm of microalgae to the pollutants.

Table 2,
levels of the parameters studied that divided by statistical program.

Run No.	Temp °C	pH	$\frac{wastewater}{algae\ broth}$ ratio
1	20	5	1
2	20	6	1.5
3	20	7	2
4	20	8	2.5
5	25	5	1.5
6	25	6	1
7	25	7	2.5
8	25	8	2
9	30	5	2
10	30	6	2.5
11	30	7	1
12	30	8	1.5
13	35	5	2.5
14	35	6	2
15	35	7	1.5
16	35	8	1

Table 3 shows the results of the pollutants concentration and their removal per cent according to the parameters of table 2.

Table 3,
Effect of parameters of table 2 on the concentration of pollutants and their removal per cent for sterilized wastewater using broth of microalgae and treatment time of 48 hours.

Run No.	TOC Conc. mg/L	TOC removal %	COD Conc.mm g/L	COD removal%	NO_3 Conc.mm g/L	NO_3 removal %	PO_4 Conc.mm g/L	PO_4 removal%
Control	24.4	0	132	0	2.3	0	9.84	0
1	4.587	81.2	29.96	77.3	1.104	52.0	1.673	83.0
2	6.32	74.1	35.25	73.3	1.134	50.7	2.135	78.3
3	8.2	66.4	50.16	62.0	1.214	47.2	3.464	64.8
4	10.42	57.3	61.78	53.2	1.35	41.3	3.87	60.7
5	5.5	77.5	31.95	75.8	1.143	50.3	1.93	80.4
6	3.074	87.4	30.1	77.2	1.093	52.5	1.28	87.0
7	9.03	63.0	59.4	55.0	1.334	42.0	3.64	63.0
8	7.174	70.6	50.95	61.4	1.196	48.0	3.0	69.5
9	7.64	68.7	43.03	67.4	1.249	45.7	3.23	67.2
10	8.5	65.2	45.28	65.7	1.297	43.6	3.287	66.6
11	1.9	92.2	19.0	85.6	1.0	56.2	0.777	92.1
12	4.78	80.4	29.7	77.5	4.09	51.0	2.627	73.3
13	9.0	63.1	51.48	61.0	1.279	44.4	3.62	63.2
14	5.25	78.5	38.15	71.1	1.189	48.3	3.01	69.4
15	2.14	91.2	20.6	84.4	0.888	61.4	1.181	88.0
16	1.88	92.3	19.54	85.2	0.8	65.2	0.67	93.2

It can be seen from table 3 that the maximum %removal of pollutants TOC = 92.3%, NO_3 = 65.2%, and PO_4 = 93.2% all at experiment number 16 (T = 35^{o}C, pH 8, and (wastewater/algae broth) ratio = 1) while the %removal of pollutant COD = 85.6% at experiment 11 (T = 30^{o}C, pH 7, and (wastewater/algae broth) ratio = 1).

The %removal of the pollutants above may be greater by about 5% if the unsterilized wastewater is used.

From table 3 it is clear that the ratio of wastewater/algae broth has greater effect on the reduction of pollutants compare with the temperature and pH.

In general, the reduction of pollutants is increased with increasing of temperature, neutral of pH and decrease of wastewater/algae ratio.

4. Statistical Analysis

By statistical analysis, table 4 shows the coefficients for each predicted equation of the pollutants removal per cent.

Table 4,
Coefficients for each equation of the predicted value for pollutants removal per cent.

Pollutant = bo+b1*T+b2*pH+b3*r+b4*T*pH+b5*T*r+b6*pH*r+b7*T*pH*r
T = Temperature, r = wastewater/(Algae broth) ratio

Coefficient	TOC	COD	NO_3	PO_4
bo	2.33194	28.80407	104.6561	109.6277
b1	4.96731	2.61733	-1.8757	0.4482
b2	11.06484	5.80584	-11.2473	-7.8307
b3	30.29022	44.49052	-14.9886	-6.5373
b4	-0.58434	-0.24116	0.4613	0.1807
b5	-2.49989	-2.11445	0.4295	-0.5944
b6	-6.93836	-8.02477	2.9692	1.2390
b7	0.37222	0.29222	-0.1311	-0.0078
% Error from actual	R^2 = 0.991	R^2 = 0.973	R^2 = 0.953	R^2 = 0.96

From table 4, It can be seen that the parameter has more effect on the treatment is (wastewater/ (Algae broth) ratio, while the other parameters have less effect in their range.

5. Adsorption Isotherm

The type of the adsorption for pollutants on the organic microalgae are analyzed using three types of adsorption isotherms [13, 14, 15]:

- Linear Isotherm: $C_S = KC$ …(1)
- Freundlich Isotherm: $C_S = K_F\, C^{1/n}$ ...(2)
- Langmuir Isotherm: $C_S = \dfrac{C_{S\,\max} C}{K_L + C}$ ...(3)

Where:
C_S = equilibrium solute concentration per unit amount of adsorbent (kg solute/m^3 solid)
C = equilibrium solute concentration in solution (kg/m^3)
K = linear equilibrium constant (-)
K_F = Freundlich adsorption constant
The dimensions of K_F depend on the dimensions of C_S and C and the value of n.
K_L = Langmuir adsorption constant, it has the same dimensions of C.
C_{Smax} = maximum concentration of solute (pollutant) on the solid (microalgae)

The equilibrium data of C_S and C that occur during the experiment will follow any of the adsorption isotherms above when the relation between C_S and C is linear as shown in the checking below:

For check the linearity a linear adsorption isotherm C_s is plot against C (Eq. 1), for Freundlich adsorption isotherm, using linear form of Eq. 2 ($\log C_S = \log K_F + \frac{1}{n}\log C$) $log\ C_s$ is plot against $log\ C$ and for Langmuir adsorption isotherm also using linear form of Eq.3 ($\frac{1}{C_S} = \frac{K_L}{C_{Smax} C} + \frac{1}{C_{Smax}}$) $1/C_s$ is plot against $1/C$ (Eq. 3).
It is taken 1 – 4 experiments of the table 3 when the wastewater/ (algae broth) ratio change from 1 – 2.5. Table 5 shows the equilibrium concentrations of pollutants in the wastewater (C) and on the algae (C_S). The cell density of *Chlorrela vulgaris* was determined by measuring the optical density of a 10-mL sample at 682 nm by using UV-vis spectrophotometer (UV 1800, Shimadzu Scientific Instruments)

Table 5,
Equilibrium concentrations in the wastewater and on the microalgae for pollutants.

Run No.	Dry weight of algae g	TOC		COD		NO_3		PO_4	
		C mg/L	*Cs mg/g*	*C mg/L*	*Cs mg/g*	*C mg/L*	*Cs mg/g*	*C mg/L*	*Cs mg/g*
1	2.3	4.587	8.614	29.96	44.36	1.104	0.52	1.673	3.55
2	1.53	6.32	11.817	35.25	63.24	1.134	0.762	2.135	5.036
3	1.15	8.2	14.087	50.16	71.17	1.214	0.944	3.464	5.544
4	0.92	10.42	15.2	61.78	76.33	1.35	1.032	3.87	6.49

Figures (22 –24) show the relation between C_S and C for the pollutant TOC.

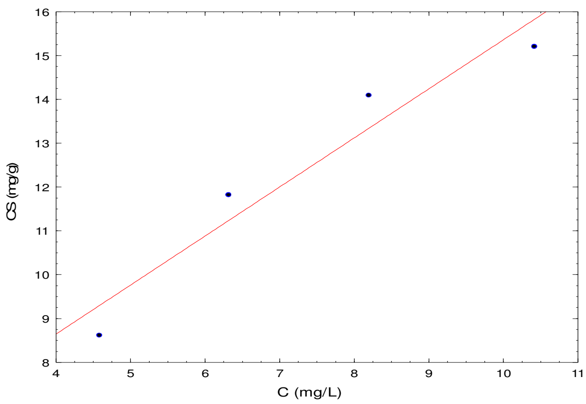

Fig. 22. Plot the concentrations according to linear adsorption isotherm for TOC.

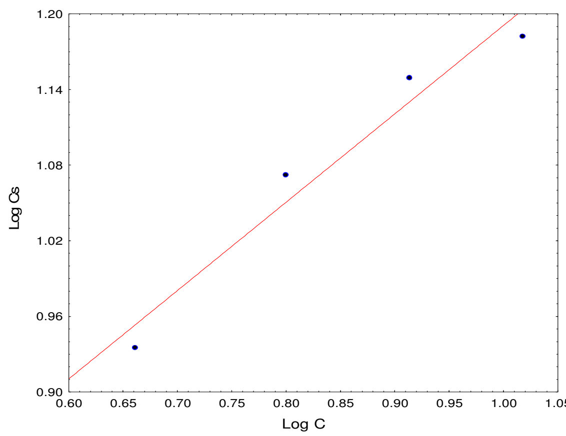

Fig. 23. Plot the concentrations according to Freundlich adsorption isotherm for TOC.

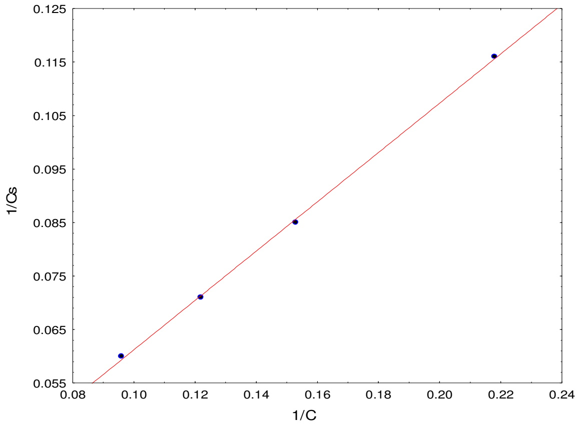

Fig. 24. plot the concentrations according to Langmuir adsorption isotherm for TOC.

It is clear that from figure 24 the data concentration of TOC are agreement with Langmuir adsorption isotherm compare with figure 22 (Linear isotherm) and figure 23 (Freundlich isotherm).

By the same way, it can be test the adsorption isotherm for the other pollutants and found that they are also agreement with Langmuir adsorption isotherm as shown in the figures 25 – 27 for COD, NO_3 and PO_4 respectively.

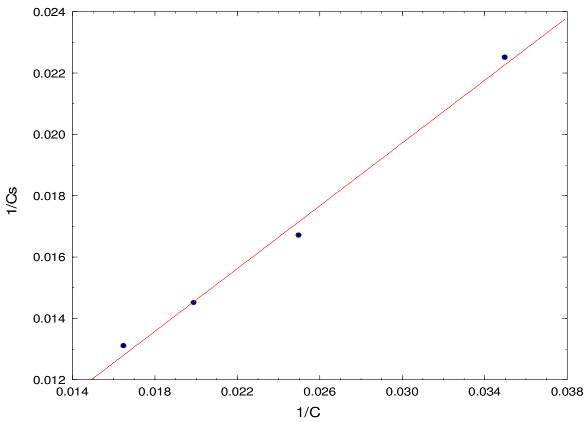

Fig. 25. Plot the concentrations according to Langmuir adsorption isotherm for COD.

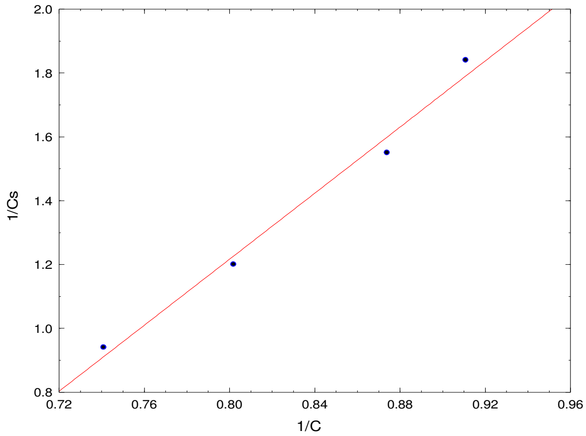

Fig. 26. plot the concentrations according to Langmuir adsorption isotherm for NO_3.

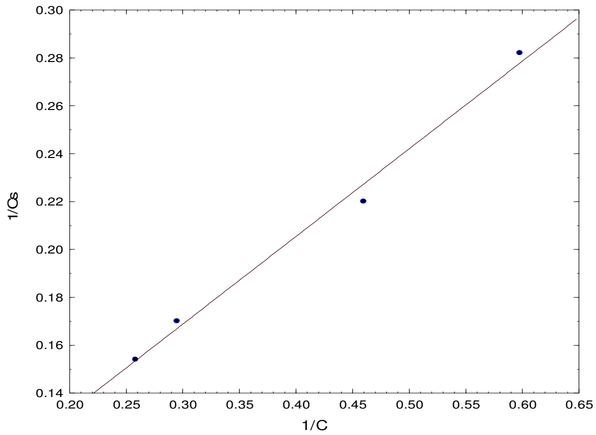

Fig. 27. Plot the concentrations according to Langmuir adsorption isotherm for PO_4.

From figures 24 the constants of the Langmuir Isotherm (C_{Smax} and K_L) for all pollutants are estimated and shown in table 6:

From equation 3;

$$\frac{1}{C_S} = \frac{K_L}{C_{Smax}C} + \frac{1}{C_{Smax}} \quad \ldots(4)$$

Where the K_L/C_{Smax} is the slope and $1/C_{Smax}$ is the intercept of the figure 24 and other figures that are drawn according to the Langmuir Isotherm.

Table 6,
Constants of Langmuir isotherm for all treated pollutants.

Constants	TOC	COD	NO_3	PO_4
C_{Smax} mg/g	47.62	232.56	1.742	15.38
K_L mg/L	19.63	127.33	10.05	5.71

6. Conclusions

1. The reduction of pollutants in unsterilized wastewater is more effective than the sterilized wastewater.
2. For the sterilized wastewater the maximum removal of the pollutants that achieved after 48 hr are: TOC = 92.3%, COD = 85.6%, NO3 = 65.2%, and PO4 = 93.2% and can be obtain more reduction by about 5% if the unsterilized wastewater is used.
3. The reduction of pollutants is very fast in first hours of treatment and progressively slows down.
4. Broth of microalgae (without packing) has more efficiency than the microalgae that grown on the packing and the packing of cylindrical plastic has more efficiency than the cubic polystyrene.
5. The temperature and pH have little effect on the reduction of pollutants, while the volume ratio of wastewater/algae broth has the great effective on the treatment.
6. The adsorption of all pollutants that treated (TOC, COD, NO3, and PO4) are followed the Langmuir isotherm.

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خفض الملوثات من مياه فضلات البلدية بأستخدام الطحالب المجهرية Chlorella vulgaris

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الخلاصة

في هذا البحث تم خفض ملوثات مياه صرف البلدية باستخدام الطحالب المجهرية Chlorella vulgaris. الملوثات التي تم معالجتها هي: مجموع المركبات الكربونية (TOC)، الطلب على الأوكسجين الكيميائي (COD)، النترات (NO_3)، والفوسفات (PO_4). أُنجزت المعالجة انجزت في الظروف الجوية الأعتيادية (درجة الحرارة = ٢٥ درجة مئوية) ، درجة الحموضة ٧ مع الوقت (١- ٤٨ ساعة). لدراسة تأثير الكائنات الحية الدقيقة الأخرى على الحد من الملوثات ، تم استخدام مياه الصرف الصحي المعقم ومياه الصرف غير المعقمة لنوعين من الحشوات (بلاستيك أسطواني الشكل وبوليستيرين مكعبة الشكل) وكذلك مرق الطحالب (بدون حشوة) ، حيث تنمى الطحالب على الحشوات ثم تنقل إلى مياه الصرف الصحي للمعالجة. وأظهرت النتائج أن الكائنات الحية الدقيقة الأخرى في مياه الصرف غير المعقمة من الممكن أن تساهم بصورة قليلة في المعالجة. تبين أن الحشوات البلاستيكية اسطوانية الشكل أكثر فعالية من البوليستيرين المكعب ، كما أن حساء الطحالب يعطي نتائج أفضل من الطحالب المزروعة على الحشوات لكلا النوعين أعلاه. كانت المعالجة في الساعات الأولى سريعة بينما كانت المعالجة للساعات الأخيرة بطيئة للغاية. بعد ذلك تم دراسة المؤثرات الأتية في نطاق (درجة الحرارة: ٢٠– ٣٥ °م) درجة الحموضة (٥ - ٨) ، ونسبة الحجم من مياه الصرف الصحي إلى مرق الطحالب (١ - ٢,٥) حيث استخدم مياه صرف معقمة وعند زمن معالجة يساوي ٤٨ ساعة. أظهرت النتائج أن الحد الأقصى لخفض الملوثات هو: TOC = ٩٢,٣٪، NO_3 = ٦٥,٢ ٪ ، PO_4 = ٩٣,٢٪ عند درجة حرارة = ٣٥ °م، درجة حموضة ٨ ، ونسبة (مياه الصرف الصحي/ مرق الطحالب) = ١، و COD= ٨٥,٦ ٪ عند درجة حرارة = ٣٠ °م، درجة حموضة ٧ ، ونسبة (مياه الصرف الصحي/ مرق الطحالب) = ١. درجة الحرارة ودرجة الحموضة لها تأثير قليل على خفض الملوثات مقارنة مع نسبة مياه الصرف الصحي/ مرق الطحالب. كذلك تمت دراسة علاقة الأمتزاز للملوثات لثلاثة أنواع من الأمتزاز؛ Linear، Freundlich و Langmuir. وأظهرت النتائج أن جميع الملوثات التي تم علاجها تتبع علاقة Langmuir الامتزازية.