THE USE OF LOCAL BLUE-GREEN ALGAE IN THE BIOREMEDIATION OF HYDROCARBON POLLUTANTS IN WASTEWATER FROM OIL REFINERIES

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Abstract

Five blue green algae species were isolated (*Westiellopsis prolific*, *Anabaena variabilia*, *Oscillatoria pranceps*, *Phormidium mucicola* and *Lyngbya digueti*) from the Iraqi aquatic environment. These algal-isolates were tested for reduction the hydrocarbons pollution waste which represented Alcohol, Alkanes, Alkynes, Cyclo Alcohol, Cycloalkanes, Carboxylic Acid, Dehydrated. Three algal – enzymes were detected amylase, protease and beta-lactamase, which play important role on the hydrocarbon concentrations reduction. *P. mucicola* recorded removal ratios (40.57, 59.57, 74.28 and 85.28%) with treatment period of 24, 48, 72 and 96 hours, respectively. The exponential increase of biomass for five species were 1.5, 1.613, 2.218, 2.889 and 3.913 mg / l. from wet weight 1.74, 2.86, 3.30 and 3.87 g / l. which their contributed for increasing the degradation ratios respectively. While the *O. pranceps* recorded removal ratios 26.42, 61.71, 77.57 and 92.85% in line with the increase of biomass 1.98, 2.31, 2.67 and 3.177 g / L respectively. Moreover, the removal ratios were 26.42, 61.71, 77.57 and 92.85% by use the algal biomass 1.98, 2.31, 2.67 and 3.177 g / l. from *O. pranceps*. The *A. variabilia* ability appeared for removal ratios 35.71, 52.57, 77.14 and 87.14% by use biomass 1.90, 2.217, 2.73 and 2.90 g / l. from this species. The biomass of *W. prolific* 1.95 and 2.85, and 2.320 and 3.183 g /l. which appeared removal ratios 24.28, 41.42, 71.28 and 88.57% from the hydrocarbons pollution were reduction. The optical density of algae increase during the experimental periods which tested at wavelength 540 nm.

Keywords: Bioremediation, Blue-green Algae, Enzymes, Hydrocarbon, Biomass

Introduction

Contamination of the aquatic environment with oil pollutants (hydrocarbons) is one of the most dangerous contaminants because it is toxic to most living organisms, especially aromatic hydrocarbons through its dissolved nature. Many treatments were used, including physical and chemical, such as burning and the use of emulsifiers emulsions, which reduce the surface tension between water and oil and thus work to dismantle hydrocarbons and reduce their toxicity to the neighborhoods (Plaza, 2005).

The biological treatment that uses bacteria, fungi, algae and yeast is one of the methods used to treat hydrocarbons. The treatment of the production of specialized enzymes leads to different metabolic pathways in the representation of hydrocarbons and thus decomposes the carbon chain. In addition microbiology directly connects the cell surface with a molecule Hydrocarbons are then transported through the cellular membrane. In the study of (Zhang and Miller, 1994), he noted that the algal cells attach themselves to the hydrocarbon droplets and that the carbon chain becomes part of the lipid the phenomenon depends on the process of binding hydrocarbons with the water-damaging surfaces of the cell. In addition, the process of bioremediation depends on a number of factors that determine the rate of hydrolysis of hydrocarbons, including (oxygen, temperature, microbiology, growth conditions, carbon chain length, nutrient salts) which accelerate the growth of microorganism to generate enough surface area to absorb and treat oil pollutants (Anton *et al*., 2000.) Bio-treatment from other traditional treatment methods is characterized by many advantages, being low-cost, eliminating pollution without leaving traces or residues of the pollutant, and dismantling pollutants into their natural end products in the environment (Atlas, 1981) and (NIST, 2016), so the study aims to degradation the hydrocarbon

contaminants of the aquatic environment by uses the blue-green algae.

Materials and Methods

Isolation and cultured of algae used in treatment

Five isolates of blue-green algae were isolated from the Iraqi aquatic environment. The isolates algae were cultured by BG-11media. The algae were identified by using a Heamocytometer and examined on a 400X force using a composite optical microscope, and the references were used as classification and identification keys for the algae isolates were (Desikachary, 1959) (Edward, 2010) (Felisberto and Rodrigues, 2004) (Al-Hussieny, 2018).The concentration of algae cells was measured by using spectrophotometer at 540 nm during the experiment period (24, 48, 72 and 96) hours, the media which no cultured by algae considered as control.

Primary inoculums for algae

The size 1.5 g wet w/l used as a primary inoculums for the algae *Phormidium mucicola, Lyngbya digueti, Oscillatoria pranceps, Anabaena variabilia* and *Westiellopsis prolific*, which use for wastewater treatment in the Doura oil refinery in Baghdad.

Detection of enzymes

Five isolates were examined for producing enzymes (protease, amylase, betalactemase, acid phosphatase, and alkaline phosphatase) Each one according to the way it method.

Amylase enzyme

The maltose standard curve table (1) and Fig. (1) was prepared to detect the amylase enzyme activity in the raw extract for each sample according to method (Bernfeld, 1955).

Table 1 : Preparation of series concentrations for the stock maltose solution.

Tube no.	Maltose stock solution ml	D.W ml	Maltose concentration Mg/ml
1	0.0	1.0	0.0
2	0.1	0.9	0.2
3	0.3	0.7	0.6
4	0.5	0.5	1.0
5	0.7	0.3	1.4
6	0.8	0.2	1.6
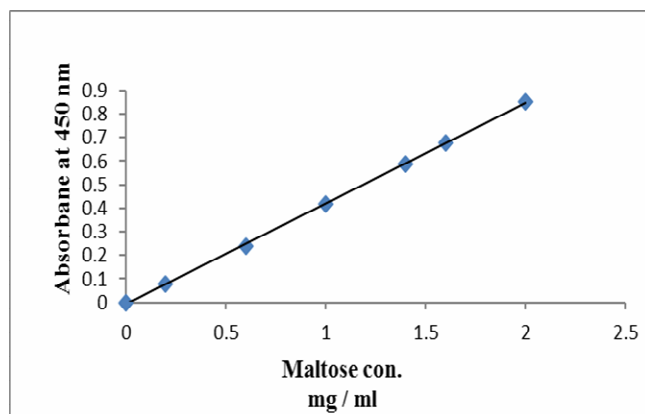
Fig. 1 : The standard curve for estimating the amylase activity extracted from algae.

Protease enzyme

The crude extract enzyme for each sample was detected for protease activity according to the method (Manachini, 1989). The enzyme activity defined as the amount of enzyme required to produce an increase in absorption at 280 nm 0.01 in one minute under experimental conditions according to the following equation:

$$\text{Enzyme activity (U/ml)} = \frac{\text{Absorbance at 280 nm}}{0.01 \times 30 \times 0.2}$$

0.01: Constant

30: Reaction time (min)

0.2: enzyme volume (ml)

Betalactemase enzyme

Detection of Bactalactime enzyme in raw extract for each sample according to method (Novick , 1962). Enzyme activity was calculated according to the following equation:

$$\text{Enzyme activity (U/ml)} = \frac{\Delta E \times 121.9}{\Delta t}$$

ΔE = blank- test

121.9: Constant

Δt : Reaction time (min)

Phosphatase

The p-nitrophenyl Phosphate standard curve table (2) and Fig. (2) was prepared to detect the phosphatase enzyme activity in the raw extract for each sample according to method (Lee, 2000).

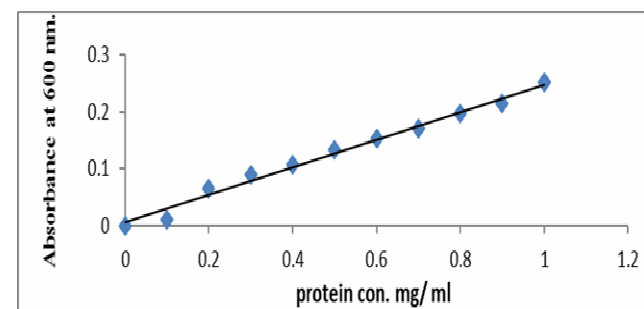**Table 2 :** Preparation of a series concentrations for the p-nitrophenyl Phosphate Stock solution

Tube no.	p-nitrophenyl Phosphate mg/ml
1	0.0
2.	0.1
3.	0.2
4.	0.4
5.	0.8
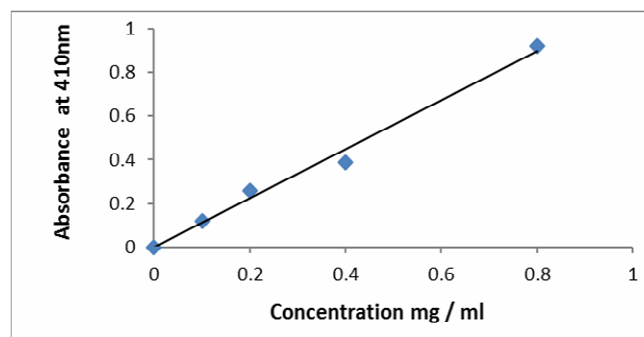
Fig. 2 : The standard curve for estimating the Phosphatase activity extracted from algae.

Protein assay

Blue green Algae were cultured within a biomass ranging from 0.0963 to 0.4945 mg/L, and used method 16 to extract algal proteins. The protein concentration was determined according to (Lowry *et al.*, 1951) method. The required concentration was prepared from the original concentration of Bovine serum albumin 100µg/ml, according to (Table 3). The relationship between absorbance and concentration was drawn to obtain protein standard curve (Figure 3)

Table 3 : Preparation of Bovine serum albumin (BSA) concentration from stock solution of BSA (100µg/ml)

Tube No.	Volume of BSA solution (ml)	Volume of D.W. (ml)	Final volume (ml)	Final concentration (µg/ml)
1	0	1.0	1	0
2	0.1	0.9	1	10
3	0.2	0.8	1	20
4	0.3	0.7	1	30
5	0.4	0.6	1	40
6	0.5	0.5	1	50
7	0.6	0.4	1	60
8	0.7	0.3	1	70
9	0.8	0.2	1	80
10	0.9	0.1	1	90
11	1.0	0	1	100

Fig. 3 : Standard curve for estimating protein (protein used for bovine serum albumine solution)

Collection of samples

Wastewater samples contaminated with hydrocarbons from the Doura power station were collected from a pre-entry stage into the biological treatment basin, which is divided into two basins of each 2050 m^3 basin containing mechanical ventilation ventilators for microbiology (bacterial and fungal) Since algae are the main product of oxygen, we do not need ventilation.

- **Detection of hydrocarbon compounds using a GC-MASS device**

Hydrocarbons were tested using the GC-MS type (2010 plus-Shimadzu) by column DB-1, thickness-0.25 mm (Maxstemp 340 ºC-30M), and according to thermal program 85 = 2 min (10C / min 300) The total in the column Total Flow 10 and Pressure 100 Kpa with the amount of sample particles entering column 5 (Toshihiro *et al.*, 2013). Five samples were taken per day (for four days of treatment) to determine which compounds were removed by the producing algae For enzymes.

- **Examination of concentrations of organic pollutants (hydrocarbons)**

Use the HORIBA oil content analyzer for hydrocarbon concentrations by following the EPA test method 413.2 as follows:

- Calibration of the device by preparing a concentration of 200 mg / L of the standard solution for the device (Bheavy oil).
- Take the form and add 1: 1 diluted HCL to the form to make acidic 2 so that we can extract all the organic material in the form.
- Pull 10 mL of the model and put it in the suppression of the chapter, and then add 10 ml of solvent (S-316) to the model and start the mixing tower of organic matter with the model.
- Leave the mixture until separated and settled, then removed the organic material on the paper filter container to 1 g of sodium sulfate anhydrous substance to absorb the moisture and water existing and descending with the organic layer in addition to the solvent.

- We put organic matter extracted in the measurement cell and read the results expressed in mg / l unit. Figure (4) shows the device used.

Fig. 4 : HORIBA oil content analyzer used to detect organic pollutants (hydrocarbons).

Hydrocarbons degradation by using blue-green algae

The algae isolated include (*Phormidium mucicola, lyngbya digueti. Oscillatoria pranceps*, *anabaena variabilia* and *Westiellopsis moss*) with a optical density (0.325 nm, 0.287 nm, 0.390 nm, 0.450 nm and 0.326 nm) respectively ,were used in wastewater treatment at Al- Doura refinery which contaminated with 7 mg/l concentration of hydrocarbon oil residues), the size of the algal culture was used as a primary inoculation for 1.5 g/wet w.

Results and Discussion

Isolation and cultured of algae used for treatment

Five isolates were identification from isolated blue-green algae (*Phormidium mucicola, Lyngbya digueti, Oscillatoria pranceps, Anabaena variabilia* and *Westiellopsis prolific)* as shown in Figure (5).

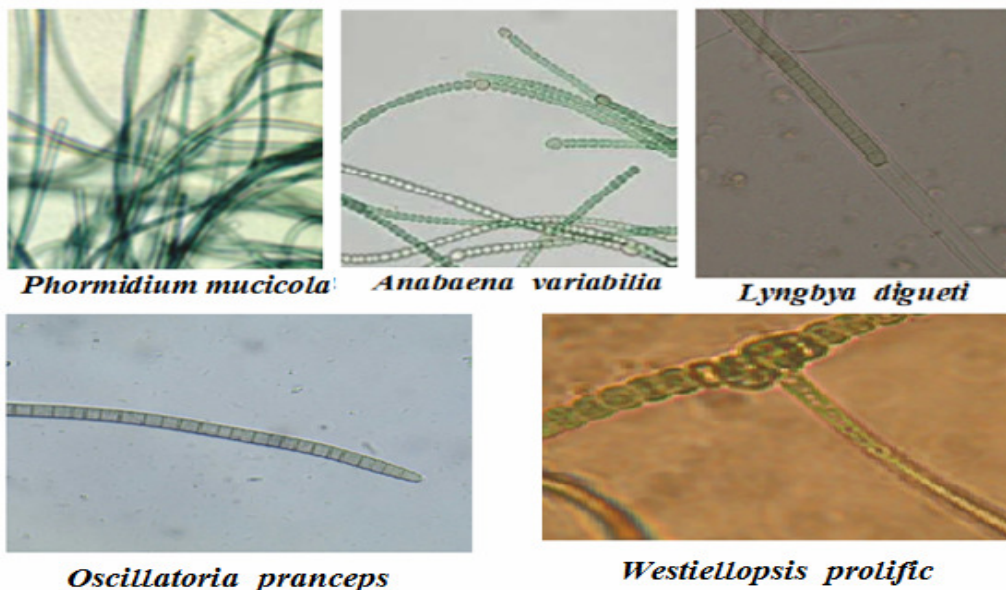**Fig. 5 :** Blue greens algae isolated from the Iraqi aquatic environment

Detection of enzymes

Five isolates of algae were used to detect the effectiveness of enzymes and proteins. The results showed in Table 1 that all isolates contained protease and amylase enzymes. The efficiency of the protease enzyme for green leaf algae was 10.115-13.3 U/The amylase enzyme was 0.112-0.562 U / ml, whereas the production of the enzyme β-alleactemase was only in the *Oscillatoria pranceps* and was effective at 26.7 U/ml, while the acid phosphetase enzyme presence in *Anabaena variabilia* and *Lyngbya digueti* with effective at 0.06 and 0.03 u/ml respectively theses results agree with (Hernández *et al*., 2002). The presence of alkaline Phosphates enzyme was not recorded in all algal isolates. a table (4) showing this.

Table 4 : Enzymae activity in some algae species

Algae species	Amylase activity u/ ml	Protease activity u/ ml	β-alleactemase u/ ml	Phosphitase activity u/ ml
Phormidium mucicola	0.372	11.783	-	-
Lyngbya digueti	0.330	12.783	-	0.03
Oscillatoria pranceps	0.114	13.3	26.7	-
Anabaena variabilia	0.447	11.766	-	0.06
Westiellopsis prolific	0.562	11.95	-	-

Studies and research indicate the role of photosynthesis in algae, which has the role of polarizing the largest amount of light to produce oxygen to help activate the enzymatic system of oxidation of hydrocarbons gave the role of algae to be treated, and through the study (Cerniglia, 1980), which tested eighteen isolates of algae Green, green, reddish, and deuterium algae in naphthalene oxidation through the oxidation system as well as metabolic products produced from algae that have a role in the decomposition of hydrocarbons (Haynes, 2015). Environmental factors play an important role in the process of treatment of hydrocarbon pollutants, including heat, salinity, oxygen, pH and salts. The importance of these factors, among other things, is the increase in the number of algal cells, thus providing a more surface area to attract hydrocarbons.

Determination of protein

The results of protein content in algal isolates ranged from 3.190 to 9.950 mg/l (Table 5). *Westiulopsis prolific* and *Phormidium mucicola* recorded the highest protein content 9.950 and 5.030 mg/L respectively, followed by *Oscillatoria pranceps*, *Lyngbya digueti* and *Anabaena variabilia* with protein content 4.940, 3.680 and 3.190 mg/L, respectively. Most algae have high protein productivity, including algal isolates in the current study. This is due to the use of the quality of the plant species involved in building the internal structure of the algal cell and containing the nutrients, As well as their focused focus With the trace nutrients, which have a key role in the structure of the internal structure, which led to an increase in the biomass of algae isolates, which consists of 40-76% of its dry weight protein (Fan, 2014).

Table 5 : Determination of protein content of green leafy algae

Algae isolates	Biomass (mg/L)	Protein (mg / L)
Phormidium mucicola	0.2347	5.030
Lyngbya digueti	0.3698	3.680
Oscillatoria pranceps	0.0963	4.940
Anabaena variabilia	0.3732	3.190
Westiellopsis prolific	0.4945	9.950

Detection of hydrocarbon compounds using GC-MASS

The results in Figure (6), showed the presence several types of hydrocarbon compounds (Aromatic hydrocarbons, Alcohol, Alkanes, Alkynes, Cyclo Alcohol, Cycloalkanes, Carboxylic acid, Dehydrated, Ether, Ester, Ketones and Organic Acid And Polycyclic Aromatic Hydrocarbon (PAH).
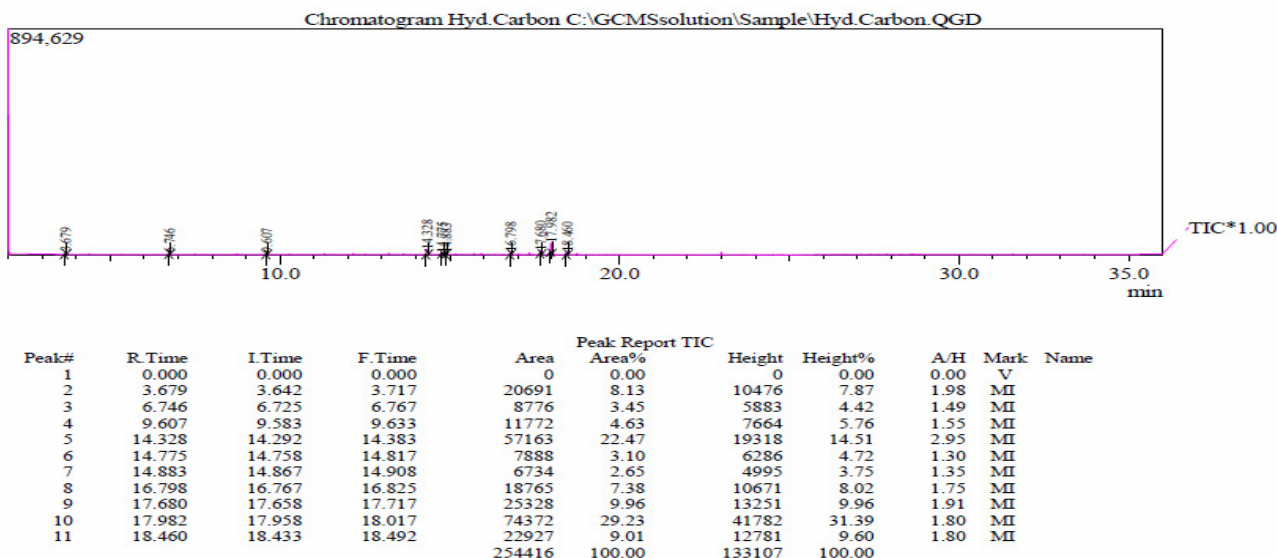

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	0.000	0.000	0.000	0	0.00	0	0.00	0.00	V	
2	3.679	3.642	3.717	20691	8.13	10476	7.87	1.98	MI	
3	6.746	6.725	6.767	8776	3.45	5883	4.42	1.49	MI	
4	9.607	9.583	9.633	11772	4.63	7664	5.76	1.55	MI	
5	14.328	14.292	14.383	57163	22.47	19318	14.51	2.95	MI	
6	14.775	14.758	14.817	7888	3.10	6286	4.72	1.30	MI	
7	14.883	14.867	14.908	6734	2.65	4995	3.75	1.35	MI	
8	16.798	16.767	16.825	18765	7.38	10671	8.02	1.75	MI	
9	17.680	17.658	17.717	25328	9.96	13251	9.96	1.91	MI	
10	17.982	17.958	18.017	74372	29.23	41782	31.39	1.80	MI	
11	18.460	18.433	18.492	22927	9.01	12781	9.60	1.80	MI	
				254416	100.00	133107	100.00			

Fig. 6 : The hydrocarbon compounds detection by GC-mass.

Hydrocarbons degradation by using blue-green algae

Researchers (Ikhimiukr and Nueji, 2013) have indicated that algae have high oxidation and decomposition capacity, including Naphthalene, and oxidation of aromatic hydrocarbons by cracking them into compounds that are less complex and simpler by enzymes and converting them into energy to feed algae, the most important of which are algae. *Microcoleus* sp., *Anabaena* sp., *Nostoc* sp., *Chlorella* sp., *Amphora* sp. and *Chlamydomonas* sp., *Ulva* sp. and

Aphanocapsa sp. This is also what the researcher pointed out (Madigan *et al.*, 2010).

Algae identified in the study were used to treat wastewater from oil refineries, the results shown in (Table 6), the treatment with algae (*Anabaena, Lyngbya digueti, Oscillatoria pranceps, Phormidium mucicola Westiellopsis prolific*) reduced the concentration of oil contaminants to 0.9, 0.7, 0.5, 1.03, 0.8 g/L respectively .

Table 6 : Reduction of hydrocarbon compounds using blue green algae

Algae isolates	Concentration of oil waste (Mg / l)		Biomass (G / L - wet weight)		Optical density (nm)	
	Before	After	Before	After	Before	After
Anabaena variabilia	7	0.9	2.90	3.073	0.052	0.068
Lyngbya digueti	7	0.7	3.23	3.87	0.022	0.031
Oscillatoria pranceps	7	0.5	3.032	3.177	0.036	0.045
Phormidium mucicola	7	1.03	3.140	3.913	0.035	0.04733
Westiellopsis prolific	7	0.8	3.004	3.183	0.026	0.034

The results showed an exponential increase in the number of *Oscillatoria pranceps* compared to control during the days of the experiment with a low concentration of hydrocarbons. The optical intensity was increasing continuously during the experimental days of the treatment compared to control. The percentage of hydrocarbons was 92.85%. 96 hours after the experiment started with the increase in biomass 3.177 g/l - wet weight (Figure 7).
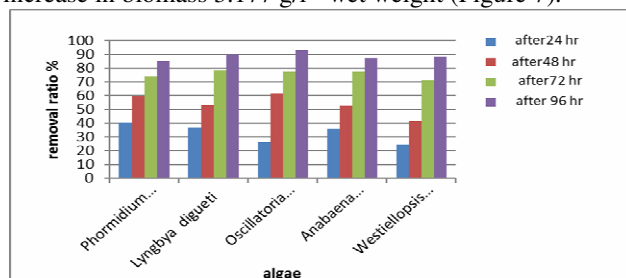

Fig. 7 : Removal ratio percentage of hydrocarbons by using blue-green algae.

The daily removal rate was 85.28% after 96 hours with the treatment by *Phormidium mucicola* which contains various enzymes including amylase, protease. The concentration of hydrocarbons was 7-1.03 mg/L with a period of 24-96 hours, while the removal rate was 88.57% with treatment by *Westiellopsis prolific* with an increase in the biomass 3.183 g/l- wet weight, respectively, this is the result due to presence of three enzymes amylase, protease and betalactemase, which reduced concentrations of hydrocarbons from 7-8 mg/L during 96 hours of experiment initiation. *Oscillatoria pranceps* were the best isolates used for reduction, which reached 92% after 96 hours of treatment, because of the possession of two enzymes (amylase and protease) and rapid movement, which controlled by the size of the medium and so it has the potential for rapid spread with an increase in biomass.

Organic pollutants in the aquatic environment are susceptible to degradation by a group of naturally occurring microorganisms, but studies have focused on the role of bacteria and fungi in treatment processes because they are capable of producing a variety of oil-soluble hydrocarbons, (Kirk, 1999) and (Werle, 2008), while the algae trends are few on this subject despite the spread of algae in most of the

water column and the basic surfaces and production of various enzymes in addition to oxygen, an important factor in the systems of disassembly of the antenna because it acts as an receiver of the electronic Data as a reference to that (Madigan *et al.*, 2010).

In recent decades, the increasing availability of biocatalysts and developments in related biochemical knowledge have led to the investigation of new sources for enzyme production. Algae have unique properties and could be explored for the large-scale production of enzymes as future biocatalyst factories. Algae enzymes and proteins are environmentally friendly and highly efficient in reducing pollutants by having rapid dispersion in surrounding areas. Nowadays, cyanobacteria and algae are viewed as increasingly attractive cell factories for producing renewable biofuels and bioactive chemicals due to their ability to capture solar energy and their relatively simple genetic background for genetic manipulation. Algae possess the advantages of low-cost production, without the need to use fresh water and high-value arable land. Moreover, they show potential as a future source of enzymes. Algae, as an extremely diverse group of photosynthetic organisms, have a wide range applications from human and animal nutrition to cosmetics and the production of high-value molecules such as fatty acids, pigments, and stable isotope biomolecules(Al-Hussieny, 2019).

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