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Investigating the effect of hydrocarbon concentration on ammonia removal by *Chlorella vulgaris* in an airlift photobioreactor

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Abstract

Environmental concerns about the contamination of groundwater and sea as a result of oil refining and transportation have encouraged scientists to seek sustainable and cost-effective methods to clean up these pollutants. Ammonia removal by green microalgae *Chlorella vulgaris* in media containing 10 mgL^{-1} ammonia and different concentrations of petroleum hydrocarbons was studied. The experiments were carried out in an airlift photobioreactor. Laboratory experiments showed that low concentrations of hydrocarbons not only did not inhibit ammonia removal but also had benefits for increasing the rates of biomass production and ammonia removal. Ammonia removal was obtained 100%. And hydrocarbon removal was obtained 100%. Based on the results obtained; microalgae utilize hydrocarbons as a carbon source. In addition, *Chlorella vulgaris* was a flexible and resistant microalgae to unfavorable conditions and quickly adapted to the low-contaminated culture solution. This feature increased the potential of *Chlorella vulgaris* to removal of ammonia and a wide range of hydrocarbons with different properties and toxicity.

Keywords: Microalgae; Ammonia; Wastewater; Airlift photobioreactor; Chlorella vulgaris

1. Introduction

Oil refining and transportation can cause considerable environmental problems and disrupt the populations of aquatic organisms. A complex mixture of various compounds, containing heavy metals, oil, grease, ammonia, alkanes, alkenes, alkynes, cycloalkanes, monoaromatics and polycyclic aromatic hydrocarbons, sulfide, phenols, sulfate, phosphate, nitrate, soluble solids, and suspended solids, is found in oily wastewater [1-6].

Recently, low-cost and nature-friendly remediation methods are the essential environmental requirements to prevent the deterioration of water quality by removing pollutants. The toxicity and variability of petroleum hydrocarbon pollutants have made them difficult to treat. Physical and chemical methods, including storage, emulsifiers, solvent extraction, UV oxidation, chlorination, chemical dispersants, and adsorbents, are used to eliminate hydrocarbons. However, these techniques are often expensive and have low efficiency [4, 5, 7-11]. The combustion of the hydrocarbons becauses the release of pollutants such as CO, CO_2, SO_x, and NO_x in the air, increasing the atmospheric temperature and warming the planet Earth.

Phycoremediation is a cost-effective method by macro or microalgae for biological degradation or removal of hazardous organic and inorganic compounds, including hydrocarbons, heavy metals, and nutrients from wastewater, industrial flue gases, soil, and air with low carbon production [12-20]. This eco-friendly technique has been present since the 1950s [21]. Due to the sensitivity of microalgae to pollutant concentration, usually, this method is usually recommended for secondary wastewater treatment systems. If the algal biomass is not contaminated with toxic and dangerous compounds, it can be use to prepare animal and human feed, biological fuels, biogas, bioethanol, and valuable byproducts [22-27]. Microalgae are credited for their potential to biodegrade crude oil or fix CO_2 from the atmosphere and discharge gases [28-31]. In the future, algae will be used as energy suppliers and small food packages in the space [32]. The absorption mechanism of algae is fascinating in some cases; for example, living and dead cells of *Selenastrum capricornutum* can absorb PAHs. The uptake rate is remarkably higher in living cells, and hydrocarbons are degraded only in living cells [33].

Usually, high concentrations of ammonia, crude oil, and hydrocarbons can reduce or stop the cell growth of algae by disturbing their metabolism. However, at low concentrations, *Chlorella vulgaris* can potentially remediate these contaminants [24, 29, 34-37]. Table 1 shows the chemical composition of this single-celled microalgae [38]. *Chlorella vulgaris* can be applied as a supplement for human and animal feed because of its high protein and nutrients.

Table 1. Chemical composition of *Chlorella vulgaris*

lipid	protein	carbohydrates	nucleic acid
14%-22%	51%-58%	12%-17%	4%-5%

Polycyclic aromatic hydrocarbons have shown high resistance to biological degradation and are considered very dangerous for human health, aquatic organisms, water resources, and the marine environment [39-42]. Moreover, *C. vulgaris* has shown resistance against the toxicity of PAHs such as fluorene, phenanthrene, naphthalene, anthracene and pyrene and is recommended for treating wastewater containing low concentrations of these compounds [43-47]. Cultivation of *C. vulgaris*, ammonia removal, and biological degradation of hydrocarbons are influenced by complex interactions among temperature, molecular arrangement and carbon chain length, ammonia concentration, oxygen, microbiology, pH, growth conditions, light, and nutrient salts [48-51]. Asghari et al. [44] investigated various concentration of fluorene on the antioxidant systems and the growth parameters in the green microalga *C. vulgaris*. The results indicated the decline of growth parameters by increasing fluorine concentrations from 10 to 50 mg/L. Moreover, it showed that *C. vulgaris* has a high ability for the biodegradation of fluorine. Abreu et al. [52] showed high production of microalgal biomass and the utilization of carbohydrate when green microalgae *C. vulgaris* was cultivated under various mixotrophic conditions.

Aslan et al. [53] investigated removal of nutrient by *C. vulgaris* at various concentrations of phosphorus and nitrogen. The results showed the quality of wastewater reduces with increasing concentration of nutrient, and the removal of nitrogen is more than phosphorus by algae culture. Park et al. [54] evaluated the ability of alga Scenedesmus for removal of nitrogen from effluent at high ammonium concentation and alkalinity. When the concentration of ammonium was at normal cultivation levels nitrate and ammonium were indistinguishable. By increasing ammonium concentration up to 100 ppm, the cell growth is continued. But at the concentration of 200–500 ppm ammonium, the cell density decreases to 70%. Bicarbonate as inorganic carbon was rapidly used that lead to cell growth. The presence of inorganic carbon is essential for ammonia removal. Cechinel e t al [55] examined Four macro-algae including Fucus spiralis, Ascophyllum nodosum, Pelvetia canaliculated, Laminaria hyperborea for metals removal from petrochemical wastewater. The wastewater had high conductivity because of various components including nickel, copper, sodium and etc. the results showed high capacity in the adsorption of different metals, and L. hyperborean showed the best performance among other brown macro-algae.

Hodges et al. [56] announced the production of 33.6 million barrels per day of wastewater of petroleum refining in 2017. One strategy was the use of microalgal for the production of high value material from petroleum refining wastewater. They investigated wastewater with nitrogen, phosphorus and suspended solids. The results showed high potential in the nitrogen, nutrients and suspended removal. Finally, they proposed production of feed, biomass-based fuels, and fertilizer as value-added products.

Kim et al. [57] investigated the removal of ammonia, phosphorus and nitrogen and the rate of biomass growth by *Chlorella sorokiniana* as heterotrophic microalgae. The result showed feed with 80 mg/L as nitrogen sources has highest phosphorus and nitrogen removal and growth rate. While the growth rate decreased by concentration decline., The phosphorus and nitrogen removal and the growth rate was higher than nitrate with ammonia as a source of nitrogen.

El-Sheekh et al. [29] investigated degradation of the crude oil by two green algae *C. vulgaris* and Scenedesmus obliquus. They showed the highest crude oil biodegradation rate at 0.5 and 1% of oil. The use of 2% crude oil showed the highest growth of *C. vulgaris* while it was obtained at 0.5% for S. obliquus at the same heterotrophic conditions.

Ma et al. [58] reported the cultivation of *C. vulgaris* in the wastewater including waste glycerol for nutrients removal from the wastewater and the production of lipids. The results show the improvement of the nutrient removal and the production of lipids from C. vulgaris. The optimal pretreated glycerol concentration was 10 g/L for *C. vulgaris* with 2.92 g/L of biomass concentration, lipid production of 163 mg/Ld, and 100% of ammonia removal.

Kumar et al. [12] reported the cultivation of microalgae *C. vulgaris* in two modes; batch and continuous of industrial flue gas and sewage wastewater (SWW). They showed high COD removal above 78% and 42% in batch and continuous mode, respectively. Moreover, other nutrients showed above 75% and 55% of removal in batch and continuous mode, respectively. The CO_2 removal was 64% and 72% in the bath and continuous mode. Both two modes indicated the highest production of biomass in mixotrophic and hetero cultivations. Kalhor et al. [24] used *C. vulgaris* for the biodegradation of petroleum hydrocarbons. The crude oil/water with concentration of 10 and 20 g/l was applied to the microalga treatment during the 7 and 14 days. The results revealed the ability of *C. vulgaris* in crude oil hydrocarbons remediation during the 14 days. Increasing crude oil concentration observed the positive effects on the algal growth, and increased dry weight of C. vulgaris.

In another study, *C. vulgaris* was used for the bioremediation of various types of PAHs [3-ring Anthracene (ANT), 2-ring Naphthalene (NAP), and 4-ring Pyrene (PYR)]. The maximum growth of C. *vulgaris* was obtained for PYR. Moreover, the lipid content declined for all PAHs treatments significantly. C. *vulgaris* showed high potential for the removal of three PAHs in about ~90–94 % ANT, ~90–92 % NAP and ~76 % PYR from the media during the 7 days [43]. Different types and configurations of open and closed culture systems and photobioreactors are designed for algae production. Large-scale stabilization or high-rate algal ponds reduce the energy required for wastewater treatment and microalgae cultivation costs, although; light penetration, low quality and the concentration of biomass production, seasonality, bacterial contamination, rapid cultivation, harvesting methods, and high evaporation losses are the main significant challenges that must overcome in open systems [59-63]. However, due to their low cost, open culture systems are an economical choice for large-scale algal cultivation.

Photobioreactors and bubbling columns are common high-efficiency and practical closed systems for algae production [64]. Airlift photobioreactor is a well-mixed reactor with uniform nutrient distribution and algal biomass recirculation. Excessive shear forces and breaking bubbles cause harmful stress and reduce biomass productivity, so the mixing rate should be continuously controlled to prevent cell destruction [65].

This type of photobioreactor has several advantages, such as the prevention of water evaporation and biomass settling, better control of some critical parameters contaning temperature, pH, light intensity and energy, CO_2 concentration, and gas exchange between the cultivation water and air, efficient heat and mass transfer, easy scale-up, high nutrient removal efficiency, the prevention of the photoinhibition and photo-oxidation, high photosynthetic efficiency, sufficient mixing for biomass production and low cost [59, 66-69]. The use of a photobioreactor including a biofilter with the aerial microalga Trentepohlia aurea for ammonium removal from wastewater showed significant capacity after the pretreatment cycle by nitrogen-free BB medium supplied with magnesium [70]. Erbland et al. [71] showed high growth of Tetraselmis chuii by photobioreactor.

Novoveská et al. [72] used Algae Systems LLC for treating above 50,000 gal/day of inlet raw municipal wastewater. A combination of aeration by photosynthetically prepared oxygen, the uptake of algae nutrients, and dewatering via flotation of suspended air rejected total phosphorus, nitrogen, and BOD with the amount of 93, 75%, and 92% from influent wastewater. Azhand et al. [73] reported the application of an airlift photobioreactor for the investigation of the effect of input gas velocity on the fixation of CO_2 by *C. vulgaris* microalgae. This study indicated the growth of *C. vulgaris* to 26.95×106 cells/mL. Moreover, CO_2 removal reached 94% at the lowest superficial gas velocity (1.88×10^{-3} m/s).

This study investigated the airlift photobioreactor for the removal of ammonia and hydrocarbons by green microalgae *Chlorella vulgaris* in media containing 10 mg/L ammonia and different concentrations of petroleum hydrocarbons.

2. Materials and Methods

2.1. Wastewater preparation

Wastewater effluent was collected from twenty different points of wastewater contaminated with ammonia and petroleum compounds. Based on the field investigations and testing of the pollutants in the evaporation ponds of the refinery, the effluent of six ponds was suitable for algae cultivation. Then ten samples were prepared with three repetitions and completely randomly in a period of 30 days. Due to algal growth in summer and winter, sampling was conducted in these seasons. The samples were stored in cool boxes (4°C) for the laboratory transportation. Due to its high resistance to the pollutants, *C. vulgaris* was observed in all locations with various concentrations of ammonia and petroleum compounds, so it was the best option for conducting our experiments. The water samples were filtered through 20µm and 50µm mesh and autoclaved for 20 minutes at 121°C to kill interfering microorganisms and spores.

2.2. Culture medium

Microalgae were cultivated in a Konvey medium containing Na_2EDTA (45 g/l), KNO_3 (100 g/l), $NaH_2PO_4.4H_2O$ (20 g/l), H_3BO_3 (33.6 g/l), $FeCl_3.6H_2O$ (1.3 g/l), $ZnCl_2$ (21 g/l), KNO_3 (100 g/l),$MnCl_2$ (0.36 g/l), $COCl_2.6H_2O$ (20 g/l), $CuSO_4.5H_2O$ (20 g/l), $(NH4)6Mo7O_2.4H_2O$ (9 g/l), Na_2SiO_3 (20 g/l), vitamin B1 and vitamin B12 (2 and 0.1 g/l, respectively).

2.3. Airlift photobioreactor

The experiments were carried out in a 20-liter airlift photobioreactor with a height of 1 m at 25 ± 1°C. The system is equipped with a temperature and pH probe. The airlift photobioreactor is shown in Figure 1. Considering the growth of algae in spring and summer, sampling was done in these seasons and at a time interval of 15 days. The physiology of the algae sampled from the ponds was examined with the help of an OLYMPUS CX-31 microscope and microalgae such as chlorella *pyrenoidosa*, *spirulina sp*., *Chlorella vulgaris*, *Oocystis pusillas*, and *Oscillatoria quadripunctulata* were observed in the wastewater, due to the observation of chlorella vulgaris colonies in the places that had the most ammonia shocks, this alga was used to conduct experiment. Microalgae cultivation is influenced by reactor configuration and light intensity [13]. Uniform distribution of light distribution intensity and temperature are required to prevent photoinhibition and photo-oxidation, therefore; fluorescent lamps around the reactor were used as the light sources to provide uniform illumination of 5000 lx for the microalgae culture with dark/light of 12:12h during the *C. vulgaris* cultivation. This type of photobioreactor has an efficient heterogeneous flow. In addition, the 2.59×10^{-3} m/s of rich air (2% V/V CO_2) provides a suitable mixing mechanism so the bubbles are distributed uniformly across the column, and the carbon dioxide is easily transferred from gas to water [52].

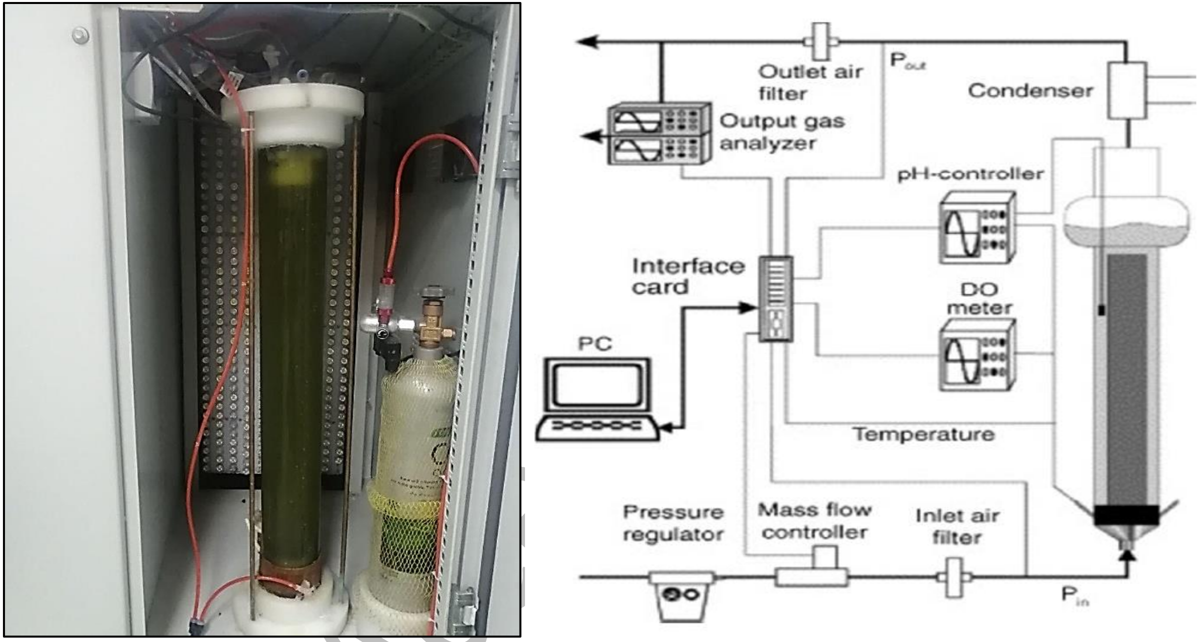

Figure 1. Schematic diagram of used airlift photobioreactor in this study.

2.4. Biodegradation estimation

The gas chromatograph with an FID detector (Agilent) with HP-5MS elastic silica capillary columns (30 m × 0.320 mm × 0.25 μm) was applied to characterize the prepared samples. The injection volume was 2 μl. Firstly, the temperature was 50 °C and then heated to 300 °C at a constant rate of 7 °C/min. GC–FID spectra were used to quantification of the compounds.

3. Results and discussion

The results showed in Table 2 are based on the analysis of four samples taken from the wastewater and show the contaminant level of petroleum hydrocarbons. Figure 2 and Figure 3 show the gas chromatograph analysis of sample one for total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs), respectively. All compositions completely disappeared in all the concentrations of crude oil incubated with algae.

Table 2. Contaminant level of TPH in wastewater

Compound	1 (µg/Lit)	2 (µg/Lit)	3 (µg/Lit)	4 (µg/Lit)
C10			146.99	3165.44
C11		182.12	323.34	533.02
C12		89.85	3409.73	698.90
C13	5.47	6.54	35.08	73.74
C14		6.02	1.54	439.75
C15		37.65	61.53	102.27
C16	2.52	29.13	104.86	55.59
C17		13.11	52.42	35.47
C18	0.56	31.39	4.29	23.34
C19	3.48	7.41	3.40	13.32
C20	0.76	1.65	0.11	7.74
C21	1.38	12.59	13.50	4.46
C22	0.77	10.82	18.22	2.01
C23	0.79	7.72	61.22	1.66
C24	0.92	6.61	2.32	0.57
C25	0.68	4.92	1.87	
C26	0.58	1.03	2.81	1.41
C27			8.28	
C28		3.37	0.94	
C29	0.67			
C30	6.04			
C31	20.20			
C32	83.22			
C33	10.51			
C34	1.80			
C35	66.26			
Fluorene	0.72			
Sum	207.34	451.92	4252.45	5158.69

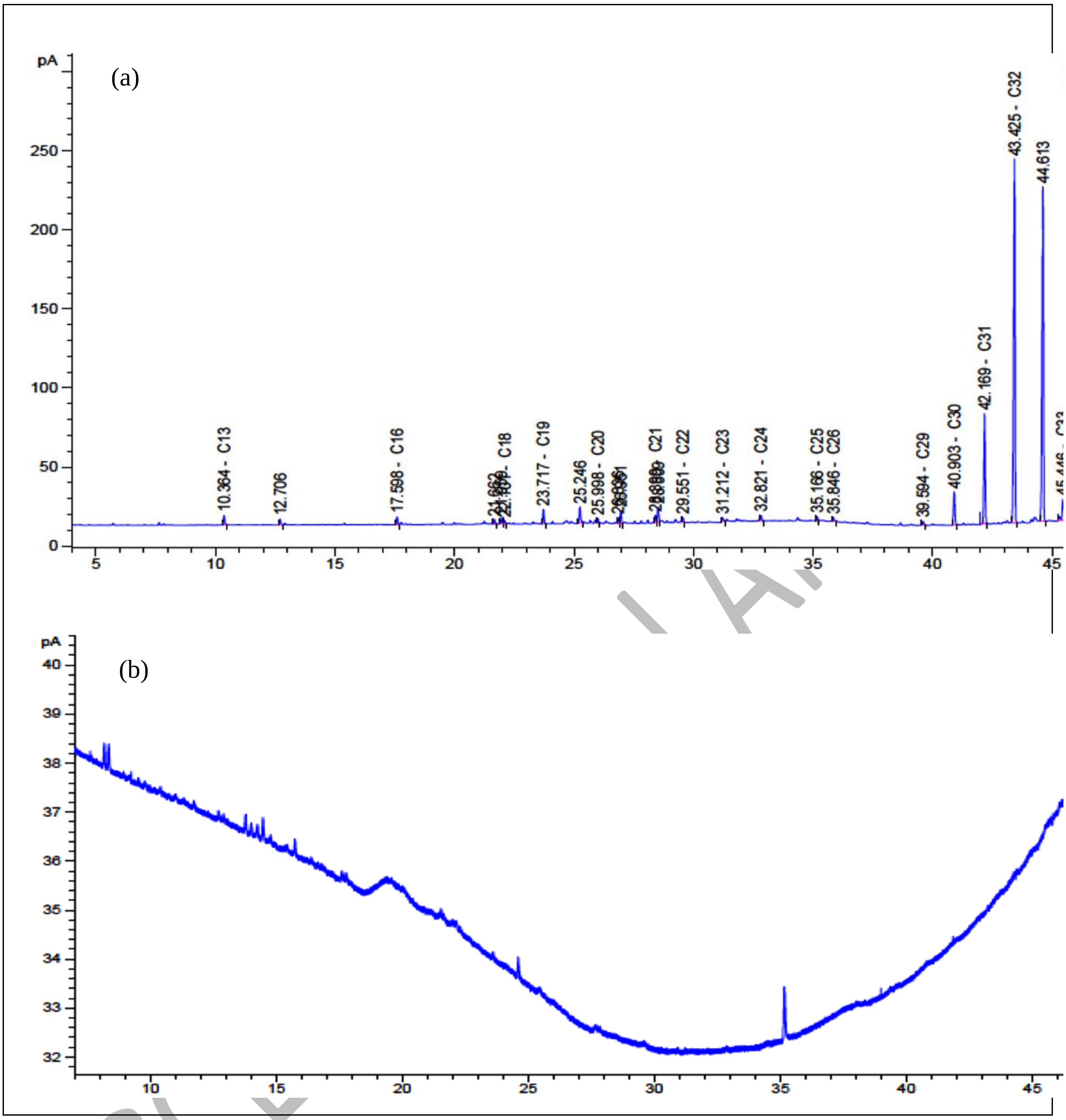

Figure 2. Gas chromatography analysis of TPH in sample one before (a) and after (b) phytoremediation.

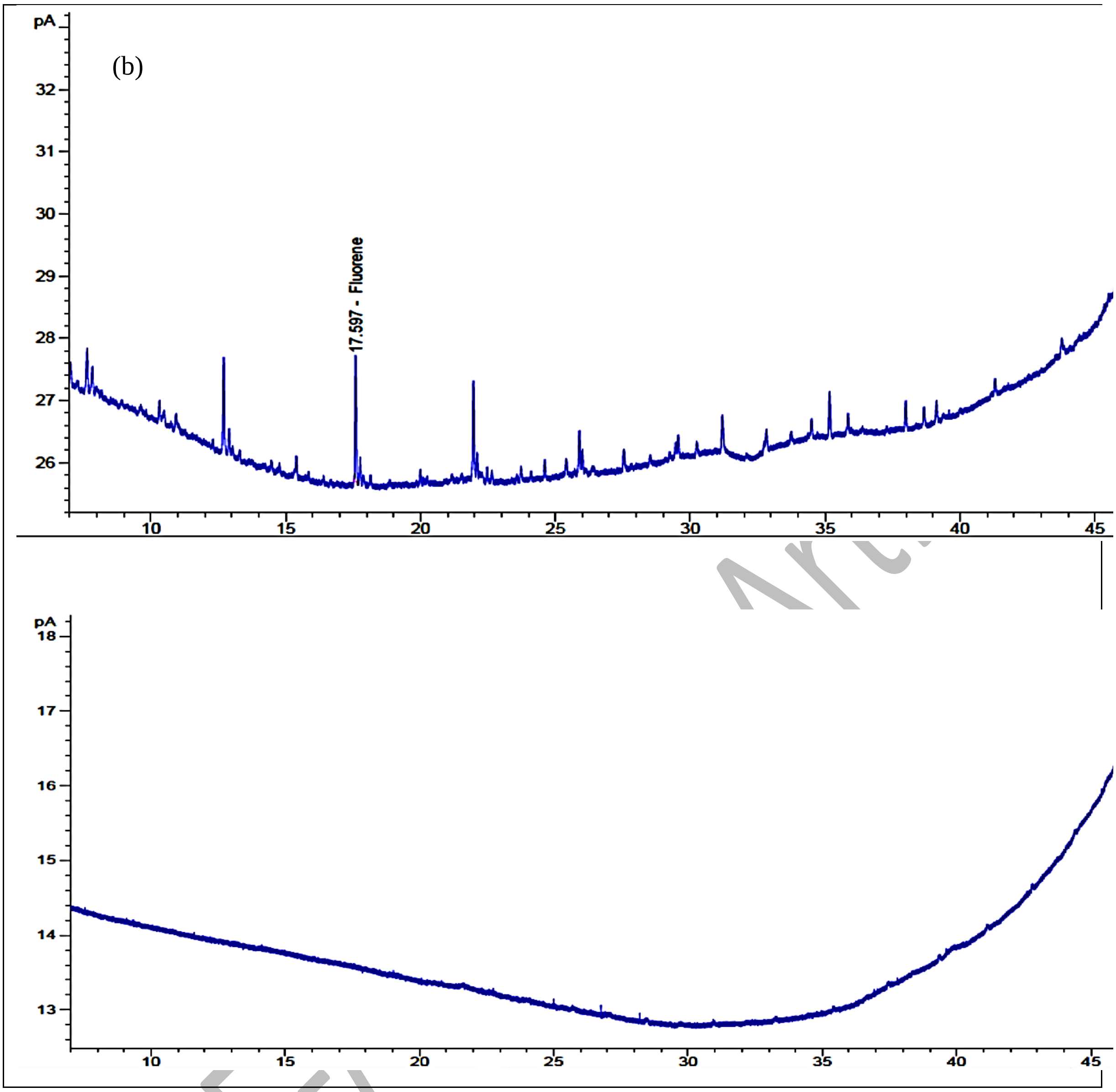

Figure 3. Gas chromatography analysis of PAHs in sample one before (a) and after (b) phytoremediation.

The results of this study indicated that the remediation process could be influenced by the initial contaminant concentrations and experimental conditions. The initial effluent containing 10 mg/l ammonia was injected into the airlift bioreactor. All experiments showed that residual ammonia decreased considerably with time, which coincided with rapid pH changes in the first 72 hours. Due to the use of an airlift photobioreactor, the effect of ammonia stripping was negligible. In addition, environmental, biological, and operational factors were controlled in optimal conditions.

If the air velocity in the photobioreactor is not adjusted correctly, the dense biomass created will interfere with absorption. Despite the low and different concentrations of hydrocarbons, the complete removal of ammonia occurs at the end of the cultivation. As shown in Figure 4. A phase lag period of 24 hours is observed at the beginning of the absorption process. total inorganic carbon uptake and *C. vulgaris* was not observed. During the phase lag, the concentration of ammonia did not decrease and the remediation of the hydrocarbons was almost equal to zero. After that, it decreased sharply with the rapid growth of phase (i.e., 24 to 120 h) and the ammonia concentration decreased to 50% after 72 hours. The performed analysis on samples NO.1 and NO.2 showed that all hydrocarbons were removed with a reasonable absorption rate at the experimental duration. However, no significant change in ammonia removal was observed due to the low concentration of pollutants. The result of other studies revealed that C. vulgaris is able in crude oil hydrocarbon remediation, and it has positive effects on the growth of the algal species [24]. The biodegradation ate was obtained 100%. While the result of degradation ate obtained 50% by El-Sheekh et al. [29] without the use of an airlift photobioreactor. The anthracene degradation by C. prothecoides in the conditions of autotrophic and heterotrophic were investigated. Degradation of anthracene was ~ 29% and 20% by light and by C. protothecoides under the autotrophic condition, respectively. Also, degradation of anthracene was 33.53% by C. protothecoides at the heterotrophic condition. C. protothecoides showed higher degradation ability and the resistance at heterotrophic condition than that at autotrophic conditions [46]. The effect of PAH such as phenanthrene (PHE) on C. vulgaris revealed significant resistance of C. vulgaris against phenanthrene as a PAH pollutant [45]. The capacity of tolerance was tested by Chlorella sorokiniana, Arthrospira platensis, Chlorella vulgaris, Arthrospira maxima (spirulina) and Tetradesmus obliquus to wastewater via increasing residue concentrations. The results observed that the decreasing iron and hydrocarbons 97.9% and75, respectively [48]. Stable cell growth requires gas velocity control to avoid shear stress, although *C. vulgaris* can tolerate unsteady conditions [73]. In the airlift photobioreactor, the photosynthesis reaction is carried out with the help of carbon dioxide, light, and inorganic nutrients. In this process, organic cell components such as proteins, lipids, nucleic acids and carbohydrates are produced. CO_2 injection from a gas cylinder provides inorganic carbon and helps us to maintain the pH at its optimal value [71]. Removal efficiency is closely related to cell density. Higher initial cell density provides more surface area for better remediation, but as the cell density increases, the culture solution becomes too viscous and impairs aeration in the airlift photobioreactor. An important factor that is very important in the proliferation of algae is the pH level of the effluent. In high and low pH values, when the culture medium becomes extremely alkaline or acidic, the living conditions for algae become difficult and the possibility of their resilience is greatly reduced [74]. Many microalgae can grow in both acidic and alkaline environments. In the studies conducted, the suitable pH range for most microalgae is reported as 7-9 [75]. Previous studies have shown that high pH increases ammonia toxicity and gradually decreases cell population. Additionally, low pH and acidic conditions inhibit biomass production. At pH 5.0 and 9.0, the algal growth efficiency decreases dramatically [50, 51, 58]. Figure 5 shows that the performance of *Chlorella vulgaris* in the ammonia adsorption process was very similar in samples NO.3 and NO.4. The phase lag decreased, and the ammonia removal rate increased simultaneously with biomass production. Moreover, the maximum cell growth of microalgae and ammonia absorption occurred in 48-120 hours, and finally all hydrocarbons were removed. Differences in hydrocarbon composition may account for the tolerance in biomass production rate. It has also been observed that in contaminated wastewater that contains higher concentrations of hydrocarbons, the carbon required by microalgae is efficiently supplied afterward, more biomass is produced, and more ammonia is removed as the surface area increases. Our findings are consistent with the results obtained in previous research.

Malfait et al. [76] showed the production of Monascus purpureus in the airlif reactor was more than stirred tank. Moreover, the opwer consuption airlift reactor was 50% mechanically srirrer reactors. Oxygen mass transfer to the aqueous phase was enhanced significantly in the airlift reactors.

Smart and et al [77] proved the possibility of continuous and extensive cultivation (within 14 days) of plant cell suspensions related to Catharanthus roseus in the small airleaf reactor with an outer ring with a volume of 0.01 m^3. Chisti et al [78] reported various types of airlift bioreactors are usful for aerobic fermentations. These reactors have very small volume compared to conventional activated sludge systems and high oxygen transfer. Mohanty et al. [79] applied multi-stage external loop airlift reactor for removal of phenol from wastewater by adsorption onto the activated carbon surface. Various parameters such as superficial gas velocity, liquid circulation velocity, contact time, pollutant concentration in wastewater, and the carbon were investigated. Using airleaf reactors to perform this separation eliminates operational complications and achieves a higher separation efficiency. Kim et al. [80] were cultivated *Aspergillius niger* in various reactors and found that the highest rate of growth algae was in the airlift reactor. Sánchez Mirón et al [81] focuced on the culturing phototrophic organisms by airlift photobioreactors. They investigated mass-transfer and hydrodynamic parameters in three air-agitated reactors: split-cylinder airlift device, bubble column and concentric draft-tube sparged airlift vessel. The results indicated that despite the difference in fluid dynamics, the production rate of *Phaeodactylum tricornutum* algae was the same in all ailrlift reactors with different designs.

S. capricornutum as a green microalgal species for degradation and removal of polycyclic aromatic hydrocarbons showed ~ 100% removal efficiency [33]. Another study showed capability of Chiarella vulgaristo to purify wastewater from different pollutant [35]. Salehi et al. [71] showed superior capacity of *Chlorella vulgaris* for hydrocarbon removal.

Light is an effective factor in algae photosynthesis. In the high depth of water, due to the lack of light penetration, the density of algae decreases drastically, because the process of photosynthesis is disturbed, and in fact, the intensity and quality of light play an important role in the growth and metabolism of algae [55]. By increasing the light intensity to the optimal level, photosynthesis and biomass production increase, but if the light intensity increases too much, photosynthesis decreases and growth stops [82].

One of the physical factors effective in the proliferation of microalgae is temperature, which is directly effective in their growth [82]. The optimum temperature for the growth of different species of microalgae is different. The most suitable temperature for the proliferation of microalgae is in the range of 15-26 [83]. Algae metabolism rate increases at higher temperatures, and low temperature is not favorable for their growth. However, other parameters such as light intensity also affect the optimal temperature [82]. Table 3 shows the comparison of ammonia removal and and biodegradation of crude oil between this study and other published studies. According to Table 3 ammonia removal and and biodegradation of crude oil are comparable to other published studies.

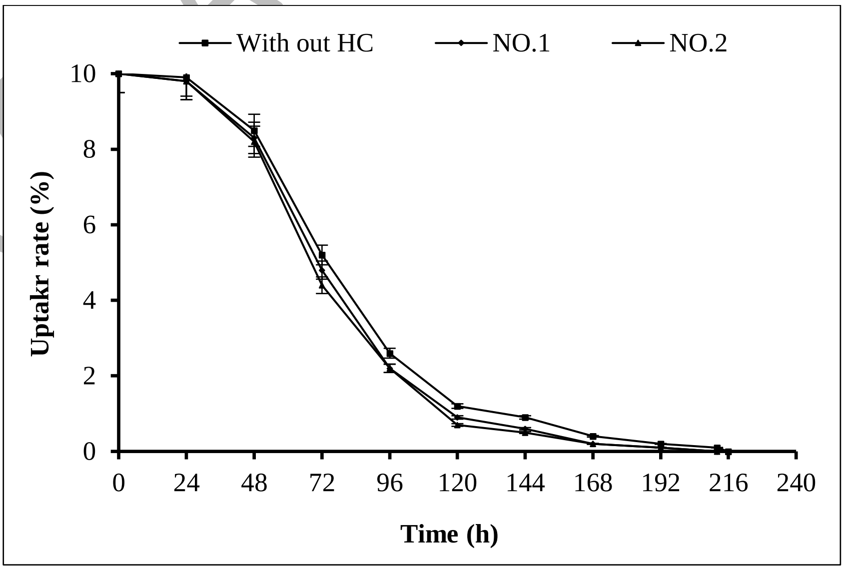

Figure. 4. The uptake rate of ammonia by *Chlorella vulgaris* with initial effluent containing 10 Mg/L ammonia

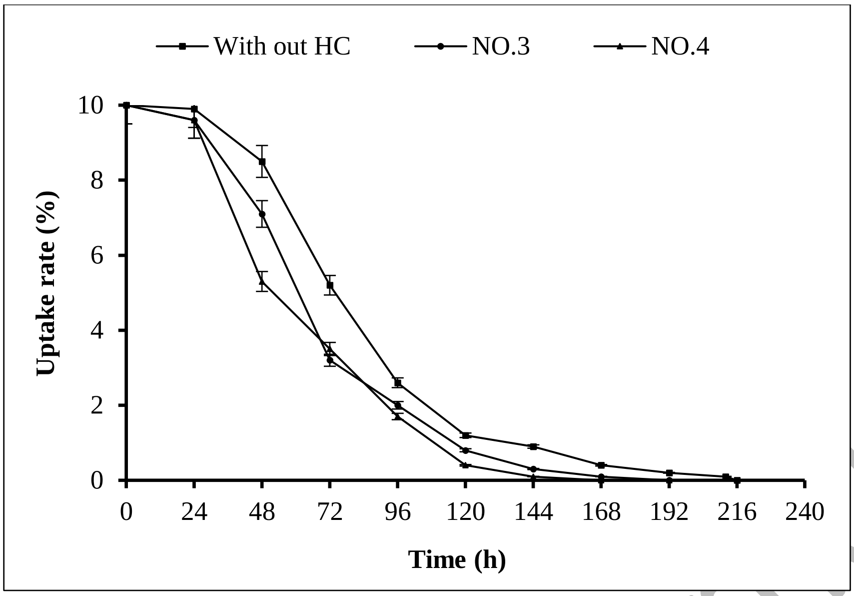

Figure 5. The uptake rate of ammonia by *Chlorella vulgaris* with initial effluent containing 10 Mg/L ammonia

Table 3. The comparison of ammonia removal and biodegradation of crude oil between this study and others.

microalgae	**Culture day**	**Initial concentration**		**Biodegradation rate**	**Ammonia removal**	**Ref.**
		amminia(mg/l)	**Hydrocarbon(mg/l)**			
C.vulgaris	14	…..	crude oil 10000, 20000	94% light compounds 88% heavy compounds	…..	[24]
C.vulgaris	7	10, 20			100	1
C.vulgaris	7	…	Naphthalene -5	90–92 %	…..	[24]
C.vulgaris	7		Anthracene -5	90–94 %		[24]
C.vulgaris	7	….	Pyrene -5	76 %	….	[24]
C.vulgaris	8	5.22–25.24		100	100	2
C.vulgaris	9	10	0.21 0.45 4.25 5.16	100	100	This study

4. Conclusions

In general, limited information is available on the toxicity of hydrocarbon and ammonia simultaneously on microalgae. The findings of this research confirmed the positive effect of hydrocarbons removal for all samples and significant ammonia removal for NO.3 and NO.4 samples in low-level polluted wastewater. Based on the results obtained, the organic compounds as a carbon source not only did not inhibit ammonia removal but also had the benefits of increasing biomass production rate and ammonia uptake. The increase in cell density and the surface area causes more contaminants absorption, so with more biomass production, ammonia removal will be increased faster and in a shorter period. In addition, *Chlorella vulgaris* is recommended as a toxicity indicator in biological wastewater treatment systems.

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