Research article

Comparative study between immobilized and suspended Chlorella sp in treatment of pollutant sites in Dhiba port Kingdom of Saudi Arabia

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ABSTRACT

Dhiba port has a strategic location near the Neom project. Various anthropogenic activities contributed to the discharge of metals, metalloids and oil spills in the aquatic system and caused environmental pollution. Microalgae are the best microorganisms in aquatic conditions known to be capable of eliminating contaminants. In this work the Chlorella sp. was isolated from seawater, the metals, metalloids were determine using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer) and hydrocarbons were determine using GC-MS in different five sites in Dhiba port, after and before treated with Chlorella sp, and immobilized Chlorella sp. The growth parameters (optical density and pigment contents) of Chlorella sp and immobilized Chlorella sp were investigated during 14 days of grown. The results showed that the most contaminated site by metals and metalloids was site no 3, by Sb, As, Be, Se, and Zn with concentrations 0.07546, 0.05709, 0.09326, 0.4618, and 0.00979 mg/L respectively, and site no 1 was the most contamination by organic compounds, so the site no 1 and site no 3 were chosen to test the efficiency of Chlorella sp. and immobilized Chlorella sp. to remove hydrocarbons and both metals and metalloids. Chlorella sp. and immobilized Chlorella sp. had completely removed metals and metalloids that were present in site 3. There were only 6 compounds remained, after treatments with immobilized alga in site 1. Immobilized Chlorella sp. is the most effective than suspended Chlorella sp in reduces the number of organic compounds in contaminated area. It is an economic tool due to simplifying harvesting and then retaining for further processing.

1. Introduction

Since coastal and maritime tourism is a new vital economic activity and pioneer in advancing the economic diversity of the Kingdom of Saudi Arabia, beaches and coastal areas' quality are based on the nearby areas' environmental quality, including ports. Ports activities of tourism and transportation, including ship discharges of ballast water, loading and unloading of cargo, and accidental discharge of oil and other chemicals in the sea have various environmental impacts that affect the extent of physiochemical and biological constituents run in the port water (Bas-tami et al., 2015; Jahan and Strezov, 2017). These impacts are significant, ranging from heavy metal contamination, oil pollution, fecal pollution to the introduction of exotic species through ballast water uptake and discharge (Luna et al., 2019; Nümi, 2004; Sany et al., 2013; Suneel et al., 2019). Due to their high toxicity to the marine environment, several studies have been examining the bioremediation of Polycyclic aromatic hydrocarbons (PAHs) and heavy metals. The term “Bioremediation” has broadly defined as the usage of microorganisms or their products to remove or eliminate pollutants. The bioremediation of contaminants in the marine environment is carried out mainly by diverse microorganisms. Algae are low-cost sorbents for the elimination of oil and can impact the fate and vehicle of spilled oil (Mishra and Mukherji, 2012). Many studies have depicted that alga eliminate nutrients such as nitrogen and phosphorus (Kim et al., 2013; Amenorfenyo et al., 2019), heavy metals (Tam et al., 2001; 11. Romero et al., 2006; Kaplan, 2013), toxic hydrocarbon, inorganic toxins, and pesticides (Abe et al., 2003; Hultberg et al., 2016; Kottuparambil and Agusti, 2018), from enclosing water by adsorption and absorption (Kozilkaya et al., 2012; El-Naggar et al., 2018) of bioaccumulation abilities of the cells (Leong and Chang, 2020). Algae bioremediation phenolics using different mechanisms such as adsorption, bioaccumulation, biodegradation, and photodegradation (Wu et al., 2022). Several species of microalgae have shown an
influential role in the remediation of both heavy metals, and hydrocarbons. Nwewe and Anebonam (2009) reported the probability of using naturally present algae isolated from a puddle near Nsukka Fire Service Station to remove hydrocarbon from water polluted with petroleum products. Microalga Chlorella kessleri could grow at different crude oil concentrations (0.5, 1, and 1.5%), mixotrophically solely and in combination with Anabaena oryzae (Hamouda et al., 2016a). Wang et al. (2018) argued that the acclimation process is a potential method of wastewater treatment using Chlorella vulgaris. C. vulgaris showed high efficiency of biodegradation under a low concentration of 0.5% and 1% of crude oil. The growth reached a high level even with the 2% of crude oil in an experiment of 15 days (El-Sheekh et al., 2013). C. vulgaris can be used for the biodegradation of crude and refined oil in contaminated aquatic environments (Samuel et al., 2020). Ankistrodesmus braunii and Scenedesmus quadricauda were able to eliminate more than 70% of phenol from olive-oil mill wastewaters within five days (Al-Dahhan et al., 2018). Hamouda et al. (2016b) reported that Scenedesmus obliquus was able to remove heavy metals Pb, Cd, Cu, and Mn, from wastewater under different conditions. Sharma and Khan (2013) noticed that Chlorella minutissima was a better efficient alga in removing heavy metals from polluted habitats than Scenedesmus spp and Nostoc muscorum. Chlorella sp. effectively removed by 76%–96% of cadmium and 78%–94% of nickel under laboratory condition (Rehman and Shakoori, 2004). Other results showed that C. vulgaris was able to remove up to 70% and tolerate 200 mg/L of As(V) present in the growth medium (Jiang et al., 2011). Immobilization of microalgae biomass simplifies biomass harvesting, contributes to the resistance of cultures against stresses, and simplifies the development of hardware for cultivation which leads to higher productivity of cultures and to an increase in the efficiency of wastewater treatment (Vasiliev et al., 2016). Moreover, the immobilization of marine microalgae could overcome the problems of high water volumes and very low concentrations of marine environments (Moreno-Garrido et al., 2005). The biomass produced during wastewater treatment may also be used to produce biofuels, bioplastics, and exopolysaccharides (Silva et al., 2022). de Jesus et al. (2019) tested the chemical stability of immobilised Desmodesmus subspicatus by counting the remaining algae over seven days of immersion in different solutions. They found that the recovery was 100% in all cases. Murujew et al. (2021) showed that recycled alginic from algae beads at a recovery rate of approximately 70% can be obtained where the recovery of alginate can bring a 60% net operational cost reduction. The disadvantages of immobilized biomass includes: added cost related to immobilization, higher mechanical diffusion resistance, and lower absorbance capacity (Blaga et al., 2021). The use of a microalgal consortium could be better than a monoculture system in terms of production and lower absorbance capacity (Blaga et al., 2021). The immobilized biomass refining includes mechanical, chemical post-harvest, mechanical or chemical disruption, or selective extraction of microalgae products and co-products (Barsanti & Gualtieri, 2018). The combination of wastewater bioremediation with the mass of microalgae improved the conventional treatment process and environmental impacts. From a bio-economy viewpoint, biofuels and value-added product recovery are important areas of technological intervention (Ummalyma et al., 2021). The main objectives of the current study are to investigate the contamination pattern in Dhiba port marine environment, analyze water in the five sites inside the port related to contaminations of heavy metals and organic carbon, and study the potential of fresh alga Chlorella sp. and immobilized Chlorella sp for possible treatment pollutants that exist in the most contaminated two sites in port. It also aims to compare between fresh alga Chlorella sp and immobilized alga for possible remediation of heavy metals and organic compounds in the most contaminated two sites.

2. Materials and methods

2.1. Sampling location and collection

The study location is Dhiba port (27° 34’ N to 34° 33’ E), located at the north-western corner of the Kingdom of Saudi Arabia. It is the nearest Saudi port to the Suez Canal and the Mediterranean basin countries’ ports, including Turkey 593 miles, Greece 491 miles, and 988 miles to the nearest French ports (Saudi Ports Authority, 2021). Thus, it acquires unique importance in its strategic location near the NEOM project, which is the Saudi Crown Prince Mohammed bin Salman’s vision and a centerpiece of Saudi Arabia’s 2030 Vision (NEOM, 2021). The registration of vessel arrivals from various ports worldwide showed that a total number of 12029 vessels had navigated the port during the period 2005–2019 (Saudi Ports Authority, 2021) (Supplementary Table S1).

Water samples were collected from the water surface on 25th January 2020 from five different Dhiba port locations (Supplementary Figure S1) in dark graduated bottles. For heavy metals and hydrocarbons determination, samples were stored in the dark at a low temperature of 4°C until examination.

2.2. Isolation and identification of Chlorella sp.

The green microalga Chlorella sp. was isolated from water samples collected from Thuwal beach, Red Sea, Saudi Arabia (22°16’35.0”N 39°05’22.3”E). The isolation was done through a serial dilution technique followed by plating on a modified BG-11 medium (Rippka, 1988; Stanier et al., 1971). The microalga identification was based on Algae Base (Guiry & Guiry, 2019), Stanier et al. (1971) and Bellinger and Sige (2015).

2.3. Preparation of immobilized microalga in alginate beads

For each flask, 30 ml of algal suspension in its exponential growth phase were harvested by centrifugation at 3000 rpm for 10 min. The supernatant was then decanted, and the volume of sediment was adjusted to 2 ml with sterilized deionized water. After that, the concentrated algal suspension was mixed with 2% (w/v) sodium alginate solution and dropped into a 2% calcium chloride solution using a sterilized burette. Beads were left to harden overnight then rinsed with distilled water.
2.4. Growth assessment

2.4.1. Optical assessment

For microalgae growth and pigments measurement, alginate beads should be dissolved in 100 ml of 0.1 M sodium citrate solution with pH 5 that was prepared by adding 10 ml of sodium citrate to a specified number of beads at 45 °C with stirring, and the beads would dissolve within one hour. Then, the solution was centrifuged at 5000 rpm for 5 min. After that, the supernatant was decanted, and the volume was adjusted to 3 ml with sterilized water. Alga’s biomass was determined every three days by measuring the algal suspension’s optical density at 600 nm using a SHIMADZU UV-2600 spectrophotometer, Japan.

2.4.2. Pigments determination

A known volume of culture was centrifuged at a speed of 3000 rpm for 10 min. After that, the algal pellets were treated with a known volume of methanol, kept in the water bath for 30 min at 55 °C, and then centrifuged again. The absorbance of the pooled extracts was registered by SHIMADZU UV-2600 spectrophotometer, Japan, at 666, 653, and 470 nm. Calculations were made according to the formulae devised by Costache et al. (2012) for chlorophyll a, chlorophyll b, and carotenoids.

2.5. The bioremediation experiment design

Two treatments were conducted triplicate to study the potential of Chlorella sp in the bioremediation of metals, metalloids, and the biodegradation of hydrocarbons. For each treatment, two Erlenmeyer flasks (250 ml) containing 150 ml of sterilized seawater were enriched with nitrogen and phosphate source (0.225 g of NaNO₃ and 0.006 g of K₂HPO₄). Under a laminar flow cabinet, three flasks were cultivated with the algal beads, and the other three were cultivated with the residue of 30 ml of centrifuged algal cells of each flask. The cultures were incubated under the conditions of 12:12h light:dark and at 25 °C temperature and slight aeration for two weeks (Supplementary Figure S2).

2.6. Chemical parameters analysis

2.6.1. Metals and metalloids

Laboratory analysis was carried out for metals (Aluminum, Barium, Cadmium, Chromium, Cobalt, Copper, Iron, Lead, Manganese, Nickel, Silver, Titanium, Vanadium, and Zinc), and metalloids (Antimony, Arsenic, Beryllium, and Selenium) were determined before and after the experiment. Metals and metalloids were measured using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer). Agilent Technologies 720 ICP-OES (Agilent Technologies Inc., Santa Clara, CA, USA). Axial. Seawater samples were filtered then diluted 10 times. No digestion needed. Calibration and its range were done with 5, 2, and 1 ppm standard solution of each metal element and were prepared in 2% nitric acid.

2.6.2. Determination of petroleum hydrocarbons

Petroleum derivatives were extracted from 100 ml of seawater of each sample. The pH was adjusted with 1 M HCl to get a pH < 3. Organic compounds were extracted via liquid-liquid phase extraction thric, using 10 ml and 5 ml of dichloromethane (CH₂Cl₂). The organic lower phase was collected, and the moisture was removed by adding about 2g anhydrous sodium sulfate (Na₂SO₄). The clear extract was transferred to a test tube and evaporated with a gentle nitrogen gas stream at room temperature. The sample concentrated to about 10 μl (Suhrhoff and Schols-Böttcher, 2016). The analysis was performed using a gas chromatograph (GCMS-QP2010 Plus, Shimadzu, Japan) equipped with a mass spectrometer with a fused-silica capillary column (30 m × 0.25 mm ID × 0.25 μm-Rtx®-1, Restek, USA) was used. Helium was used as a carrier gas, and the temperature programming was 60–300 °C, 1/5 min. GC-MS internal library search was used to identify the organic compounds. The analysis was conducted before and after the experiment.

2.7. Statistical analysis

Experiments were conducted in triplicate and expressed as ± standard error of the mean. The data were compared by analysis of variance one-way and three-way ANOVA. Significance was determined using Duncan’s multiple range tests (p ≤ 0.05). Analysis was carried out using MS Excel (2016) and SPSS (Version 16).

3. Results and discussion

The results showed the number of vessels arrived Dhiba port from ports worldwide. 12029 vessels, through 14 years ago, denoting anthropogenic activities during these years and hence accumulation of waste products (Supplementary Table S1). Results also showed nineteen heavy metals investigated in five Dhiba port sites (Supplementary Table S2).

The World Health Organization (WHO) resulting a guideline value of 3 μg/L for antimony in drinking water (WHO, 1993). The doses of arsenic in natural waters, including open ocean seawater, generally range between 1 and 2 μg/L. (Hindmarsh et al., 1986). The safe doses of beryllium concentration of 0.1 μg/L (Lytle et al., 1992). The levels of selenium in surface water range from 0.06 μg/L to about 400 μg/L (Lindberg, 1968) so the concentrations above the previous denoted the contamination. The results demonstrated different concentrations of As, Be, and Se among nineteen investigated metals. The Be and Se were found at all five sites. Site no. 1 was contaminated by Sb, As, Be, and Se with concentrations 0.03168, 0.04126, 0.08985, and 0.199 mg/L respectively where the site no. 3 was contaminated by the previous metalloid Sb, As, Be, Se, in addition to Zn metal with concentrations 0.07546, 0.05709, 0.09326, 0.4618, and 0.00979 mg/L respectively. The concentrations of metals and metalloids in surface seawaters varied from one site to another. Zinc metal has been depicted only in the third site, which has a high total concentration of metals (Ms) compared with other sites, so it was chosen for the bioremediation experiment.

The organic compounds concentrations were estimated before the experiment (Supplementary Table S3). The level of total organic compounds ranged from 0.21 ppm to 0.55 ppm. The first site was the most highly polluted with organic compounds. It showed particular compounds that were not found in the other sites (1,1,3-Trimethylcyclopentane and Diethyl Phthalate), so it was chosen for the biodegradation experiment.

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3.1. Assessment of Chlorella sp. growth

Green microalga Chlorella sp. is halotolerant, proliferating, and growing in marine environments and favorably using it for bioremediation and biodegradation experiments. Luangpipat and Chisti (2017) indicated that C. vulgaris thrived in a full-strength seawater medium and enhanced lipid productivity by nearly 2-fold compared to freshwater. Chlorella sp. is a microgreen alga that is usually found in seawater (Maghfiroh et al., 2018) C. vulgaris was cultivated in a photobioreactor with controlled conditions of NaCl that was extracted from salt from brackish and seawater (Sahle-Demessie et al., 2019) Figure 1a-b shows the suspension of Chlorella sp and Chlorella sp beads growth that was measured by optical density at 600 nm. The growth of immobilized cells reached a high level compared to fresh cells.

The immobilized cells grown in sample one reached their highest growth level close to the eighth day of cultivation, and optical density reached 2.4 nm. In the case of site 3, the maximum growth of alga beads reached at (O.D 1.8 nm) after ten days and at (O.D 0.4 nm) within seven days in the case of fresh alga. A plausible explanation for this result is that the third site was mostly contaminated with metals and metalloids as a result of the negative effect of alga growth, whereas the first site was more contaminated with organic compounds. In this case, it is preferred for alga to grow under mixotrophic conditions and use organic compounds as the sole carbon source.
Melo et al. (2018) proved that when *C. vulgaris* was grown under mixotrophic conditions, the cellular productivity increased, and it becomes more effective to remove agro-industrial by-products. The highest growth rate of *C. vulgaris* was obtained when grown under mixotrophic conditions than when grown under photoautotrophic conditions (Abreu et al., 2012). Bansal (2019) investigated whether the growth of *C. vulgaris* and *C. protothecoides* were promoted under mixotrophic conditions when using glycerol as a carbon source. When *Chlorella* spp. was grown mixotrophically on glucose, it produced superior biomass concentration than heterotrophic and photo-autotrophic conditions (Chetrislip and Torpee, 2012). The results indicated that immobilized cells were higher in growth than suspension cells in both two sites. *C. vulgaris* immobilized by sodium alginate produced a higher amount of cells than suspension cells (Abu Sepian et al., 2019; Rushan et al., 2019). The immobilization technique can offer higher micro-algal cell density, which is useful for diminishing lag period (Ide et al., 2016), due to it being less sensitive to stress conditions (Lee et al., 2020).

Results in Table 1 showed that the effect of seawater was taken for both sites on chlorophyll-a, Chlorophyll-b, and Carotenoids contents and the content of both immobilized and suspension *Chlorella* sp cells. Chlorophyll-a contents are more promote in suspension *Chlorella* sp that was grown in seawater taken from site 3 within ten days. Contaminations in site 3 were more abundant with metals and metalloid and less content of hydrocarbons, so the alga was grown under photoautotrophic conditions. Chlorophyll-a in autotrophic was promoted by alga growth, which revealed the production of necessary pigments by *Chlorella* sp for photosynthesis, the only pathway for the metabolism of phototrophic microalgae (Mohammad Mirzaie et al., 2016).

The same trends were observed for Chlorophyll-b contents but within seven days with suspended alga (Table 1). After 10 days of cultivation, Chlorophyll-a and b were decreased. This decrease may be due to the decrease in nutrient content in media such as nitrogen and phosphorus. Chlorophyll contents decrease could be due to decreasing nitrogen in media (Li et al., 2008). The highest level of carotenoid contents of *Chlorella* sp was 666.14 µg mL⁻¹ recorded on the 14th day with suspension cells grown in the seawater sample taken from site one, followed by of *Chlorella* sp that was grown on the same days but in site three. Both sites on day 14 of growth had the stress conditions such as site three that had the most contamination by metals, site one was mostly contaminated by organic compounds, and when incubations period to day 14, the nutrients of media decreased and accumulation of toxic compounds.

Green alga such as *Chlorella* can be overproducing secondary carotenoids under stress culture conditions like nitrogen limitation, cultivation period, and salt stress (Santhosh et al., 2016). A high amount of carotenoids were produced by *S. platensis* after 7 and 11 days of incubation with various concentrations of oil (El-Sheekh et al., 2013). The three-way ANOVA, shown in Table 2, demonstrated the variable among different sites, alga treatments, and the incubation periods related to Chl b, Chl a, and carotenoids. The results indicated that there was a significant interaction among sites, alga treatments (immobilized and suspended), and incubation times in relation to pigments contents in *Chlorella* sp. In site 1 there were significant interactions among the types in treatments (suspension, alga, and immobilized) and incubations periods and also in case of site three (Table 3).

### Table 1. Mean ± SEM levels of Chlorophyll-a, Chlorophyll-b, and Carotenoids contents of immobilized and suspended cells during two weeks in both sites.

|                | Immobilized cells | Suspended cells |
|----------------|-------------------|-----------------|
|                | 3rd               | 7th             | 10th            | 14th            | 3rd               | 7th             | 10th            | 14th            |
| Chl-a Site 1   | 1.7590 ± 0.090ab  | 2.8603 ± 0.145ab| 0.7746 ± 0.279c | 0.7261 ± 0.279c | 3.8327 ± 1.000de | 1.6714 ± 0.069ef| 2.7945 ± 0.145ef| 2.9531 ± 1.000ef|
| Site 2         | 1.0438 ± 0.271b   | 1.4280 ± 1.000e | 1.7205 ± 0.069d | 0.9944 ± 0.271b | 3.5895 ± 1.000f  | 2.3724 ± 1.000de| 8.6548 ± 1.000de| 1.8361 ± 0.090bc|
| Chl-b Site 1   | 3.3528 ± 0.376d   | 3.5713 ± 1.000e | 1.4355 ± 1.000f | 0.5768 ± 1.000f | 5.4971 ± 1.000f  | 4.1711 ± 1.000f | 4.3690 ± 1.000f | 2.3913 ± 1.000f |
| Site 2         | 1.8529 ± 1.000f   | 2.6673 ± 1.000e | 3.2932 ± 1.000f | 0.2538 ± 1.000f | 6.2402 ± 1.000f  | 7.2719 ± 1.000f | 2.60 ± 1.000f  | 1.2563 ± 1.000f |
| Car Site 1     | 2.20 ± 1.000f     | 47.43 ± 1.000e  | 97.3699 ± 1.000f| 78.9526 ± 1.000f| 426.77 ± 1.000f  | 276.65 ± 1.000f | 298.87 ± 0.428f| 666.14 ± 1.000f |
| Site 2         | 137.04 ± 1.000f   | 171.18 ± 1.000e | 213.34 ± 1.000f | 106.36 ± 1.000f | 417.44 ± 1.000f  | 297.05 ± 0.428f| 479.23 ± 1.000f| 555.77 ± 1.000f |

aValues in the same column with different letters are significantly different at p < 0.05 according to three-way ANOVA followed by Duncan’s test.

Figure 1. Growth curves of immobilized (A) and suspended (B) *Chlorella* sp cells measured as optical density 600 nm. (a) Growth on sample one for metals and metalloid bioremediation experiment. (b) Growth on sample three for organic compounds biodegradation experiment.
Li et al. (2019), who studied the biotreatment of mixed wastewaters with MnO₂ industry by *C. vulgaris*. However, heavy metals (Cu, Cr, Pb, and Cd) were removed from dyes by *C. vulgaris* was significantly enhanced when endophytic bacterial strain MN17 inoculum was applied (Mubashar et al., 2020). Marine green alga *Chlorella* sp. NKG16014 exhibited the highest elimination of Cd due to cell adsorption and intracellular accumulation (Matsunaga et al., 1999). Sorption capacities of heavy metals such as Cu, Zn, Cd, and Ni by *C. vulgaris* were attained at the lowest biomass concentration (Abdel-Hameed, 2010) The metals and metalloids in the current study’s contamination levels can be correlated to contamination caused by the port activities.

### 3.3. The biodegradation of petroleum hydrocarbons

Results in Supplementary Table S3 investigated the organic compounds that were existent in five sites in Dhiba port. The results demonstrated that site no. 1 was much contaminated by hydrocarbons, so it was shown for applied *Chlorella* sp and immobilized *Chlorella* sp for possible bioremediation and cleaning. Results in figures 2a, b, and Table 4 revealed the effect of *Chlorella* sp and immobilized *Chlorella* sp on removing organic compounds that exhibited in site one. Both treatments were effective in the biodegradation of hydrocarbons but the highest biodegradation rate of organic compounds was observed with immobilized *Chlorella* sp. Muñoz et al. (2003) suggested that the microalgae release biosurfactants that could improve phenanthrene degradation. Madadi et al. (2016) recommended using *C. vulgaris* and surfactants to treat wastewaters from petroleum industries. *C. vulgaris* had a high ability in remediation of crude oil hydrocarbons within 14 days (Xaaldi Kalhor et al., 2017). The results showed a complete absence of the previous hydrocarbons and a presence of new compounds. These new compounds may be due to the conversion of hydrocarbons into intermediate compounds (Okoh, 2006). This result is in agreement with El-Sheekh et al. (2013) who proved the ability of the *C. vulgaris* to degrade n-alkane and PAHs. Several studies established the vital role of *C. vulgaris* in the biodegradation of PAHs in the ecosystem (Abdel-Shafy and Mansour 2016; Wang and Zhao 2007).

Results indicated that immobilized *Chlorella* sp was more efficient to degrade organic compounds. This cells immobilization technology would accelerate the nutrient uptake rate of microalgae for improving the efficiency of seawater treatment. Immobilized *Chlorella* sp cells under optimal

### Figure 2.

GC/MS chromatogram of residual organic compounds after 14 days of incubation. (a) with immobilized *Chlorella* sp. cells. (b) with suspended *Chlorella* sp. cells.
Table 4. Concentrations in ppm of organic compounds after experiment with both suspended and immobilized *Chlorella* sp. cells.

| Compound Name                        | Molecular formula | Suspended *Chlorella* sp. | Immobilized *Chlorella* sp. |
|--------------------------------------|-------------------|--------------------------|-----------------------------|
| 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | C_{17}H_{26}O_{3} | 8.054702                 | 2.845451                    |
| 1-Docosene                           | C_{22}H_{44}      | ND                       | 3.64665302                  |
| 9-Octodecanamide (2-)               | C_{18}H_{36}NO    | 15.82382                 | 6.32248652                  |
| Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | C_{16}H_{30}O_{5} | 37.79498                 | 3.058358                    |
| Hexatriacontane                      | C_{36}H_{72}      | 20.76870683              | 7.992108                    |
| Tetrapentacontane                    | C_{40}H_{84}      | 40.48991343              | 3.985724123                 |
| Tetracontane                         | C_{36}H_{70}      | 8.444043                 | ND                          |
| n-Heptadecanol-1                     | C_{17}H_{34}O     | 5.708477                 | ND                          |
| Octacosanol                          | C_{36}H_{72}O     | 8.281868                 | ND                          |
| 13-Docosanamide                      | C_{24}H_{50}NO    | 26.94995                 | ND                          |
| Tetracosane                          | C_{40}H_{82}      | 5.985861                 | ND                          |
| Octadecaconic acid, 2,3-di-hydroxypropyl ester | C_{22}H_{48}O_{4} | 14.97093                 | ND                          |

* ND-Not detected.

conditions are effectively efficient in eliminating nonylphenol from contaminated water (Gao et al., 2011). Liu et al. (2012) reported that immobilized *Chlorella sorokiniana* GXNN 01 was vital species for use in wastewater treatment. Immobilized *C. vulgaris* was capable of removing NH4 and N from wastewater (Fraile et al., 2005). Immobilized cells have amplified reaction rates due to superior cell density (Mallick, 2002).

4. Conclusions

*Chlorella* sp. was the most effective in removing heavy metals that existed in two sites than suspension alga, there are many intermediate compounds were found after treatments by both immobilized and fresh alga, but the number of compounds were less than found in water treatments. Harvesting beads from media is very simple, and could be applied in biofuel production after bioremediation processes. It should be repeated study every year on port at different sites that represent the port activates, used different algae, and many factors effects in bioremediation processes.

Declarations

**Author contribution statement**

Ragaa Hamouda: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Abrar Alhumairi: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Amna Saddiq: Contributed reagents, materials, analysis tools or data.

Data included in article/supp. material/referenced in article.

**Declaration of interest’s statement**

The authors declare no conflict of interest.

**Additional information**

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**References**

Abdel-Hameed, M., 2010. Effect of algal density in bead, bead size and bead concentrations on wastewater nutrient removal. Afr. J. Biotechnol. (ISSN: 1684-5315) 6 (10), 6.

Abdel-Shafy, H.I., Manour, M.S.M., 2016. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egyptian J. Petroleum 25 (1), 107–123.

Abe, K., Matsumura, I., Imamaki, A., Hirano, M., 2003. Removal of inorganic nitrogen sources from water by the algal biofilm of the microalga Trentepohlia aurora. World J. Microbiol. Biotechnol. 19 (3), 325–328.

Abreu, A.P., Fernandes, B., Vicente, A.A., Teixeira, J., Dragone, G., 2012. Mixotrophic cultivation of Chlorella vulgaris using industrial dairy waste as organic carbon source. Bioresources. Technol. 118, 61–66.

Abu Sepian, N.R., Mat Yasin, N.H., Zainal, N., Rushan, N.H., Ahmad, A.L., 2019. Fatty acid profile from Immobilized Chlorella vulgaris cells in different matrices. Environ. Technol. 40 (9), 1110–1117.

Al-Dahhan, M., Al-Ani, F., Al-Saned, A., 2018. Biodegradation of phenolic components in wastewater by micro algae: a review. MATEC Web Conf. 162, 05009.

Amenofeny, D.K., Huang, X., Zhang, Y., Zeng, Q., Zhang, N., Ben, J., Huang, Q., 2019. Algal bioremediation: potentials, benefits, and the challenges. Int. J. Environ. Res. Publ. Health 16 (11).

Bansal, S., 2019. Mixotrophic growth of *Chlorella* sp. Using glycerol for the production of biodiesel: a review. Mapana – J. Sci. 18 (2), 1–12.

Barsani, L., Guattieri, F., 2018. Is exploitation of microalgae economically and energetically sustainable? Algal Res. 31, 107–115.

Bastami, K.D., Neyestani, M.R., Shemirani, F., Soltani, F., Haghparast, S., Akbari, A., 2015. Heavy metal pollution assessment in relation to sediment properties in the coastal sediments of the southern Caspian Sea. Mar. Pollut. Bull. 92 (1–2), 237–243.

Beacham, T.A., Sweet, J.B., Allen, M.J., 2017. Large scale cultivation of genetically modified *Raphidocelis subcapitata* and *Chlamydomonas reinhardtii* for biodiesel production. Int. J. Environ. Res. Publ. Health 14 (11), 123.

Benasla, A., Hausler, R., 2021. A two-step cultivation strategy for high biomass production and lipid accumulation of *Chlorella vulgaris*. Biomass 1 (2), 94–104.

Blaga, A.C., Zaharia, C., Sotu, D., 2021. Polyacrylamides as support for microbial biomass-based adsorbents with applications in removal of heavy metals and dyes. Polymers 13 (17), 2893.

Chandra, R., Iqbal, H.M., Mulla, F., Lee, H.S., Nagra, S., 2019. Algal biorefinery: a sustainable approach to valorize algal-based biomass towards multiple product recovery. Bioresources. Technol. 278, 346–359.

Cheesil, R., Torpee, S., 2012. Enhanced growth and lipid production of microalgae under mixotrophic culture condition: effect of light intensity, glucose concentration and fed-batch cultivation. Bioresources. Technol. 110, 510–516.

Costache, M., Campeanu, G., Neata, G., 2012. Studies concerning the extraction of chlorophyll and total carotenoids from vegetables. Roman. Biotechnol. Lett. 17, 7702–7708.
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de Jesus, G.C., Bastos, R.G., da Silva, M.A., 2019. Production and characterization of alginate beads for growth of immobilized Desmodesmus subspicatus and its potential to remove potassium, sodium and nitrates from sugarcane vinasse. BioCatag. Agric. Biotechnol. 22, 101438.

Dąbrowski, M., Krzemieniewski, M., Zielinski, M., Kazimierowicz, J., 2021. Immobilized microalgae-based photobioreactor for CO2 capture (IMP-CO2PBr): efficiency estimation, technological parameters, and prototype concept. Atmosphere 13 (8), 1001.

El-Naggar, N.E.-A., Hamouda, R.A., Mousa, I.E., Abdel-Hamid, M.S., Rabei, N.H., 2018. Removal of heavy metals and production of bioethanol by green alga Scenedesmus obliquus growing under heterotrophic conditions. Int. Biodeterior. Biodegrad. 122, 128–134.

Hamouda, R.A., Yeheia, D.S., Hussein, M.H., Hamzah, H.A., 2016b. Removal of heavy metals and production of bioethanol by green alga Scenedesmus obliquus grown in heterotrophic wastewater. Sains Anal. 13, 47–56.

Hamid, J.T., McCurdy, R.F., Savory, J., 1986. Critical and environmental aspects of arsenic toxicity. CRC Crit. Rev. Clin. Lab. Sci. 23 (4), 315–385.

Mishra, P.K., Mukherji, S., 2012. Biosorption of diesel and lubricating oil on algal biomass. 3 Biotechnol. 2 (4), 301–310.

Momin,Mirzae,M.A.,Kalkani,M.,Moussavi,M.S.,Ghobadian,B.,2016. Investigation of mixotrophic, heterotrophic and autotrophic growth of Chlorella vulgaris under agricultural waste medium. Prep. Biochem. Biotechnol. 46 (2), 150–156.

Moreno-Garrido, I., Campana, O., Lubin, I.M., Blasco, J., 2005. Calcium alginate immobilized microalgae: experiments on growth and short-term heavy metal accumulation. Mar. Pollut. Bull. 51 (8–12), 823–829.

Muhammad Mirzaie, M.A., Kalbasi, M., Moussavi, S.M., Ghobadian, B., 2016. Investigation of mixotrophic, heterotrophic and autotrophic growth of Chlorella vulgaris under agricultural waste medium. Prep. Biochem. Biotechnol. 46 (2), 150–156.

Guiry, M.D., Guiry, G.M., 2019. AlgaeBase. World-Wide Electronic Publication, National University of Ireland, Galway. http://www.algaebase.org.

Hamouda, R.A.E.F., Sorour, N.M., Yehia, D.S., 2016a. Biodegradation of crude oil by Anaeraba orenii. Chlorella kessleri and its consortium under mixotrophic conditions. Int. Biodeterior. Biodegrad. 122, 118–128.

Hamouda, R.A., Yehia, D.S., Hanan, M.H., Hamza, H.A., 2016b. Removal of heavy metals and production of bioethanol by green alga Scenedesmus obliquus grown in heterotrophic wastewater. Sains Anal. 13, 47–56.

Hamid, J.T., McCurdy, R.F., Savory, J., 1986. Critical and environmental aspects of arsenic toxicity. CRC Crit. Rev. Clin. Lab. Sci. 23 (4), 315–385.

Kottuparambil, S., Agusti, S., 2018. PAHs sensitivity of picophytoplankton populations in tropical coastal waters off Kozhikode, India. Environ. Health Prev. Med. 23 (1), 61–75.

Gao, Q.T., Wang, Y.S., Tan, N.F.Y., 2011. Removal and biosorption of ninyophenol by immobilized Chlorella vulgaris. Bioreour. Technol. 102 (22), 10230–10238.

Garcia del Pino, M., Oliveira, C., Tognotti, L., Cervello, A., 2017. Effects of equilibrium, thermodynamic, and kinetic study. J. Dispersion Sci. Technol. 38 (3), 368–375.

Hamid, J.T., McCurdy, R.F., Savory, J., 1986. Critical and environmental aspects of arsenic toxicity. CRC Crit. Rev. Clin. Lab. Sci. 23 (4), 315–385.

Kaplan, D., 2013. Absorption and adsorption of heavy metals by microalgae. Handb. Biotechnol. Water Purif. Wastew. 1, 327–347.

Kassim, M.A., Latif, N.A.F.A., Hashim, N.H.F., 2018. Decolorization and total nitrogen estimation, technological parameters, and prototype concept. Atmosphere 12 (8), 214–228.

Kaplan, D., 2013. Absorption and adsorption of heavy metals by microalgae. Handb. Biotechnol. Water Purif. Wastew. 1, 327–347.

Kwak, S.M., 2018. Microalgae immobilization for bioethanol production and heavy metal removal from aqueous solutions. Sci. Rep. 8 (1), 12456.

Mishra, P.K., Mukherji, S., 2012. Biosorption of diesel and lubricating oil on algal biomass. 3 Biotechnol. 2 (4), 301–310.

Momin,Mirzae,M.A.,Kalkani,M.,Moussavi,M.S.,Ghobadian,B.,2016. Investigation of mixotrophic, heterotrophic and autotrophic growth of Chlorella vulgaris under agricultural waste medium. Prep. Biochem. Biotechnol. 46 (2), 150–156.

Moreno-Garrido, I., Campana, O., Lubin, I.M., Blasco, J., 2005. Calcium alginate immobilized microalgae: experiments on growth and short-term heavy metal accumulation. Mar. Pollut. Bull. 51 (8–12), 823–829.

Muhammad Mirzaie, M.A., Kalbasi, M., Moussavi, S.M., Ghobadian, B., 2016. Investigation of mixotrophic, heterotrophic and autotrophic growth of Chlorella vulgaris under agricultural waste medium. Prep. Biochem. Biotechnol. 46 (2), 150–156.

Niimi, A.J., 2004. Role of container vessels in the introduction of exotic species. Mar. Pollut. Bull. 49 (9–10), 778–782.

Nwee, N., Aniebonam, C., 2009. Bioremediation of petroleum products impacted seawater using locally available algae. Bio-Research 7 (1), 329–328.

Jian, S., Strezov, V., 2017. Water quality assessment of Australian ports using water quality evaluation indices. PLoS One 12 (12), e0198284.

Jiang, Y., Purchase, D., Jones, H., Garelick, H., 2011. Technical note: effects of arsenate toxicity. CRC Crit. Rev. Clin. Lab. Sci. 23 (4), 315–385.

Pottapramangal, S., Agusti, S., 2018. PAHs sensitivity of picophytoplankton populations in the Red Sea. Environ. Sci. Pollut. 25 (4), 470–476.

Lee, H., Jeong, J., Im, S., Jang, A., 2020. Optimization of alginate bead size immobilized with Chlorella vulgaris and Chlamydomonas reinhardtii for nutrient removal. Bioreour. Technol. 302, 122891.

Lee, Y., Lee, Y., Han, S.H., Hwang, S.J., 2013. The effects of wavelength and wavelength mixing ratios on microalgae growth and nitrogen, phosphorus removal from Scenedesmus sp. for wastewater treatment. Bioreour. Technol. 130, 75–80.

Kasahara, K., Terao, T., 2017. Bioadsorption of Cu(II) and Pb(II) ions from industrial wastewater using novel mesoporous biochars. Water Res. 111, 114–121.

Kato, S., Tani, K., Ang, Y., 2017. Effects of inorganic nitrogen sources on growth and production of glutathione (GSH) and phytochelatins (PCS) in Chlorella vulgaris. Int. J. Phytoremediation 19 (3), 283–294.

Kaplan, D., 2013. Absorption and adsorption of heavy metals by microalgae. Handb. Biotechnol. Water Purif. Wastew. 1, 327–347.

Kasahara, K., Terao, T., 2017. Bioadsorption of Cu(II) and Pb(II) ions from industrial wastewater using novel mesoporous biochars. Water Res. 111, 114–121.

Kawaroe, M., 2018. Identification of the aggl1 mutation responsible for negative phototaxis in a wild-type strain of Chlamydomonas reinhardtii. Biochem. Biophys. Rep. 7 (1–2), 279–285.

Jian, S., Strezov, V., 2017. Water quality assessment of Australian ports using water quality evaluation indices. PLoS One 12 (12), e0198284.

Jiang, Y., Purchase, D., Jones, H., Garelick, H., 2011. Technical note: effects of arsenate toxicity. CRC Crit. Rev. Clin. Lab. Sci. 23 (4), 315–385.
Wang, L., Wang, H., Chen, X., Zhuang, Y., Yu, Z., Zhou, T., 2018. Acclimation process of cultivating Chlorella vulgaris in toxic excess sludge extract and its response mechanism. Sci. Total Environ. 628–629, 858–869.
Wang, X.-C., Zhao, H.-M., 2007. Uptake and biodegradation of polycyclic aromatic hydrocarbons by marine seaweed. J. Coast Res. 1056–1061. http://www.jstor.org/stable/26481736.
WHO (World Health Organization), 1993. Guidelines for drinking-water quality. In: Recommendations. Geneva, Switzerland, 2nd edition. World Health Organization.
Wu, P, Zhang, Z., Luo, Y., Bai, Y., Fan, J., 2022. Bioremediation of phenolic pollutants by algal-current status and challenges. Bioreour. Technol. 350, 126930.
Xaaldi Kalhor, A., Movafeghi, A., Mohammadi-Nassab, A.D., Abedi, E., Bahrami, A., 2017. Potential of the green alga Chlorella vulgaris for biodegradation of crude oil hydrocarbons. Mar. Pollut. Bull. 123 (1–2), 286–290.
Zou, H., Huang, J.-C., Zhou, C., He, S., Zhou, W., 2020. Mutual effects of selenium and chromium on their removal by Chlorella vulgaris and associated toxicity. Sci. Total Environ. 724, 138219.