

Exploring the Effects of Microalgal Biomass on the Oil Behavior in A Sand-Water System

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ABSTRACT

Exploring the Effects of Microalgal Biomass on the Oil Behavior in A Sand-Water System

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This study focused on the impact of microalgal biomass on the oil behavior in a sand-water system. The microalgal biomass was characterized and the interaction between microalgal biomass and oil was analyzed through FTIR. The effects of different conditions including microalgal biomass dose, pH, temperature and salinity on the oil behavior were investigated. A two-level factorial analysis was also used to further explore the interactions of these conditions. The microalgal biomass was found to be the most influential parameter for the residual crude oil on sand. Higher microalgal biomass dose resulted in less residual oil on sand. The remaining oil decreased with increasing solution pH from 4 to 7, and an increase of remaining oil was observed when the pH was further increased above 7. In addition, temperature and salinity could affect the removal of crude oil in the presence of microalgal biomass. Increasing the temperature up to 30 °C could result in less residual oil on sand compared with that at 5 °C. High salinity attributed to more oil removal from contaminated sand was more favorable than that at low salinity. Different mechanisms are related to oil removal and the behaviors of oil in this sand-water system could be influenced by the solution chemistry. The results of this study indicate that the presence of algae in the oiled shoreline can be considered in the comprehensive evaluation of spill risk and prediction of oil fate.

TABLE OF CONTENTS

TABLE OF TABLES	vi
TABLE OF FIGURES	vii
LIST OF ABBREVIATIONS.....	viii
CHAPTER 1. INTRODUCTION.....	1
CHAPTER 2. LITERATURE REVIEW.....	4
2.1 Oil spill and response	4
2.2 Characteristics of biomass.....	6
2.3 Removal of petroleum pollutants from water using algae-derived adsorbents.....	11
2.3.1 Removal of crude oil using algae-derived adsorbents.....	11
2.3.2 Removal of PAHs using algae-derived adsorbents	13
2.4 Removal of petroleum pollutants from water using adsorbents derived from fungi, and shells	17
2.4.1 Removal of oil using adsorbents derived from fungus, bacteria and shells	17
2.4.2 Removal of PAHs using adsorbents derived from fungus, bacteria and shells.....	18
2.5 Major factors which can impact the process	20
CHAPTER 3. MATERIALS AND METHOD	23
3.1 Materials.....	23
3.2 Experimental procedure	24
3.3 Factorial design	24
3.4 Analytical methods.....	28
3.5 Quality assurance/quality control.....	29
CHAPTER 4. RESULTS AND DISCUSSION	31
4.1 Characterization of microalgal biomass interacted with oil.....	31
4.2 Effect of microalgal biomass dose on oil behavior	35

4.3	Effect of pH on oil behavior.....	36
4.4	Effect of temperature on oil behavior	38
4.5	Effect of salinity on oil behavior.....	39
4.6	Factorial analysis of environmental conditions and its implications	40
CHAPTER 5. CONCLUSIONS.....		46
5.1	Summary	46
5.2	Recommendation for future study.....	46
REFERENCES		48

TABLE OF TABLES

Table 2-1 Use different types of algae-derived adsorbents in the removal of petroleum pollutants	16
Table 3-1 Sand specification used in this research	23
Table 3-2 Typical Characterization of crude oil	23
Table 3-3 Coded and uncoded values of the factors.	25
Table 3-4 Coded levels and corresponding values for factorial design matrix.....	26
Table 4-1 Wavenumber (cm^{-1}) and assignment of the major bands of ATR-FTIR spectra of crude.....	33
Table 4-2 Estimated effects and coefficients for residual oil remained on sand	42

TABLE OF FIGURES

Figure 2-1 SEM images of (a) alga <i>Ulva lactuca</i> (Ibrahim et al., 2016), (b) dried <i>Chlorella vulgaris</i> (El-Sheekh et al., 2019), (c) <i>E. intestinalis</i> biomass (Boleydei et al., 2018)	8
Figure 2-2 SEM images of (a) surface of heat dried fungus <i>A. malaysianum</i> biomass (Majumder et al., 2017), (b) dried flake of bacterial cellulose (<i>Gluconacetobacter sucrofermentans</i>) (Atykyan et al., 2020), (c) crab shell powder (Cai et al., 2019)	10
Figure 2-3 Adsorption, chelation/complexation and surface precipitation are different processes involved in biosorption	12
Figure 2-4 Sketch picture of bacterial chemotactic biosorption (Meng et al., 2019)	18
Figure 4-1 (A) SEM image of <i>C. vulgaris</i> cell, (B) Energy Dispersive X-Ray Spectroscopy (EDS) of <i>C. vulgaris</i> cell, (C) Particle size analysis of <i>C. vulgaris</i> biomass	32
Figure 4-2 ATR-FTIR spectra for crude oil, algae, and crude oil * algae (A). Microscopic imaging (B1, B2) and FTIR spectromicroscopic imaging for samples of algae, and crude oil * algae at 3001-2765 cm ⁻¹ (C1, C2), 1490 – 1326 cm ⁻¹ (D1, D2), 1721 – 1694 cm ⁻¹ (E1, E2)	34
Figure 4-3 The effect of biomass dose on the oil behavior in a sand-water system.	Error!
Bookmark not defined.	
Figure 4-4 The effect of pH on the oil behavior in a sand-water system.....	37
Figure 4-5 The effect of temperature on oil behavior in a sand-water system	39
Figure 4-6 The effect salinity on oil behavior in a sand-water system.....	40
Figure 4-7 Pareto chart for standardized effects for Residual oil remained on sand	43
Figure 4-8 (A-D) Main plot for four factors, (E-J) interaction effect plot for each two factors...	45

LIST OF ABBREVIATIONS

ANOVA	One-way analysis of variance
BaP	Benzopyrene
DI	Deionized
EDX	Energy Dispersive X-ray
FTIR	Fourier Transform Infra-Red
HOC	Hydrophobic organic compound
NAP	Naphthalene
OPA	Oil particle aggregate
PAH	Polycyclic aromatic hydrocarbon
PHE	Phenanthrene
PYR	Pyrene
SEM	Scanning Electron Microscopy
XPS	X-ray Photo Electron Spectroscopy
UV-Vis	Ultraviolet-visible
XPS	Energy Dispersive X-ray
XRD	X-ray Diffraction

CHAPTER 1. INTRODUCTION

One of the most important threats to the ocean ecosystem is oil spills. Most marine oil spills can be resulted from accidental leakage from supertankers and pipelines, oil drilling and some natural events (An et al. 2017). Pushed by winds and currents, the spilled oil can often reach the shoreline, resulting in environmental and economic damage eventually (Chen et al. 2019). Such oil may strand at the high tide line, leaving black lines or globs of oil on the beach as the tide recedes. Rocks, beaches, or plants can coated by thick layer of oil, while small amount of oil can even remain in some shorelines for decades. For most spills on shoreline, natural removal and weathering processes could remove the majority of stranded oil within months or a few years. There are specific conditions which may delay normal physical removal or weathering processes (Owens et al. 2008). In recent years, some studies have been conducted to investigate the oil fate and behaviors in the shoreline contaminated by oil spill (Khan et al. 2004).

The complex patterns of water movement close to coasts tend to concentrate oil in certain areas. The fate of oil is dependent on various factors such as shoreline topography and composition, the exposure of oil to wave and tidal energy, and the characteristics of oil when it arrives (Sharma et al. 2020). The physical transport of spilled oil from the sea surface to the ocean bottom can be mediated by oil particle interaction (Muschenheim and Lee 2002). Suspended particulate matter and aggregates of detritus, living organisms and inorganic matter known as marine snow, play an important role through the adsorption of hydrocarbons and the association of dispersed oil droplets (Lee 2002; Passow and Ziervogel 2016). The particles less than 2 μm with the capacity to bind hydrophobic compounds dominate free surface of natural waters, leading to a significant amount of oil sedimentation via agglomeration (Johnson et al. 2018). 87–98% of spilled oil may change to “particulate” form, either incorporated into suspended particulate matter aggregates or as particulate free oil globules (Johnson et al. 2018).

As a special type of particulates, small organisms are widespread in the marine environment. The transport of oil can also be impacted by the existence of such small organisms. For example, oil can be translocated to benthic habitats through the active feeding of zooplankton (Tao et al. 2019).

Algae are widely distributed in marine and shorelines ecosystems (Xin et al. 2020). Previous studies reported the sorption of contaminants on biomass derived from algae. The biomass obtained from saline or freshwater micro and/or macroalgae has been used as biosorbents for removing heavy metals, phenolic compounds, dyes, and aromatic hydrocarbons from water (Beolchini et al. 2006). The algal biomass contains various active sites with hydroxyl, carboxyl, and amine in their cell wall structure, making algae accessible for biosorption (Mishra and Mukherji 2012). Such structure can also result in the bonding of oil and algae (Gupta and Rastogi 2009). In the shoreline environment, algal biomass exists in both sea water and beach sediments. The presence of algae may impact the translocation of stranded oil on shoreline. Although many studies have been conducted to investigate the oil transport and fate in the marine environment, the impact of algae on the oil behaviors in contaminated shoreline has not been reported. The knowledge regarding the physiochemical nature of this impact at water-solid interface is still not available.

The passive uptake of pollutants from aqueous solutions by non-growing or non-living microbial mass can be used for the recovery and/or disposal of pollutants. The special surface properties of algae enable them to adsorb different kinds of organic pollutants from solutions. Biosorption (physical and chemical adsorption) takes place essentially in the cell wall. The main attractions of biosorption are high selectivity and efficiency, cost effectiveness and good removal performance. The raw materials which are either abundant (microalgae and sea weeds) or wastes from other industrial operations (fermentation wastes, activated sludge process wastes) can be used as biosorbents. Dead biomass cells may be stored or used for extended periods at room temperature without putrefaction. It is easy for operation and further treatment. The dead cells can show to pollutant accumulation to the same or greater extent than growing or resting cells (Aksu and Tezer 2005). Therefore, the *Chlorella* powder was used as a model in this study.

The present study aims to explore the impact of microalgal biomass on the oil behavior in a sand-water system. The microalgal biomass will be comprehensively characterized at first. The effects of shoreline environmental conditions including temperature, pH, biomass dose and salinity will be investigated. The significant factors and their interactions will be explored based on factorial analysis. These results can be used to support the risk assessment for shoreline contaminated by

oil spills. The thesis includes the comprehensive literature review on the biomass and pollutant behavior (**Error! Reference source not found.**); materials and methods for the experiments (**Error! Reference source not found.**); results and discussion (**Error! Reference source not found.**), and Conclusions (CHAPTER 5).

CHAPTER 2. LITERATURE REVIEW

2.1 Oil spill and response

Oil is one of the most important energy and raw material source for synthetic polymers and chemicals world-wide. There is risk of spillage with the potential to cause significant environmental impact whenever oil is explored, transported and stored and its derivatives are used. Pollution by petroleum oils affects many aspects such as sea life, economy, tourism and leisure activities because of the coating properties of these materials (Mokhtarian et al. 2010). When oil is spilled into a marine environment, it is subject to several processes including spreading, drifting, evaporation, dissolution, photolysis and formation of water–oil emulsions. Oil spreading is likely to occur, especially if the sea surface is still in the peculiar environment of rivers, pollutants are driven along the stream (Anderson 1997).

The majority of the stranded oil is removed for most spills within months or a few years due to natural removal and weathering processes (Tian et al. 2020). Small amounts of oil can persist for decades on some shorelines; 10-, 20-, and even 30-year old oil residues on coarse-sediment beaches have been documented for some spills. Oil spilled at sea initially spreads over the water surface as a slick a few millimeters thick. The volatile components in crude oil rapidly evaporate after spillage. This includes most of the toxic components. For example, it is estimated that at least 30% of the oil spilled by the Exxon Valdez (35,000 tonnes) evaporated into the atmosphere. As much as 40% of the Amoco Cadiz oil (240,000 tonnes) disappeared in this way. The nature of the oil is an important factor in this respect. The lighter the oil the greater the power of evaporation to remove it from the sea surface. Over half the cargo of oil spilled by the Jessica in the Galapagos in 2001 was a light oil (diesel) (Gordon Jr et al. 1973). Oil spills also harm the beauty of polluted sites and the strong odor can be felt miles away it can affect the sea color and the landscape.

Because of the action of local or tidal currents in open seas or in harbors, the consequences of pollutants are often severe. Specific conditions or circumstances may delay or prolong normal physical removal or weathering processes but the amounts of oil that persist beyond a few years represent a very small fraction of the volume that was originally stranded (Pi et al., 2019; Liu et

al., 2020a). Wind and current conspired to drive the oil slick away from the coast and the hot tropical sun caused almost all of the diesel to evaporate leaving just patches of the remaining cargo of fuel oil. It was partly for this reason that the spill was less of a disaster than originally feared. UV radiation in sunlight will oxidize some of the components present in oil, a process known as photolysis. The oxidation products include acidic and phenolic compounds, some of which may be more toxic than the original hydrocarbons (Lei et al. 2007).

Viscous oils spread more slowly than less viscous ones and therefore, water temperature, along with windspeed and sea conditions have an intense effect on the extent of oil spreading. The identification of those specific conditions or circumstances is an important component of the cleanup or treatment decision process to identify locations where it might be appropriate to remove or reduce the amount of residual oil if this is considered to be of concern for environmental or aesthetic reasons (Owens et al. 2008). As such, spreading is important in determining the fate of spilled oil through evaporation, emulsification and natural dispersion (Annunciado et al. 2005).

With the growing interest in environmental remediation, various approaches have been proposed for treating petroleum hydrocarbon contaminated sites. Among these treatment methods, soil washing has been proposed as a promising innovative remediation technology due to its potential for treating not only oil contaminated soils but also those contaminated by heavy metals (Wasko and Bratt 1991). Soil washing is less time consuming compared with bioremediation and phytoremediation, which are largely affected by climatic factors. The traditional soil washing process has been studied extensively in recent years, showing that it can be applied as an ex situ or in situ process, involving water or aqueous surfactant solutions to desorb and concentrate the contaminants into bulk liquid phase without chemically modifying them (Urum et al. 2004).

The toxicity of oil spills on contaminated soil is of a great concern especially on the environment, and this issue had been highlighted by several groups of researchers. Clearly, it could be seen that the oil spillage on soil greatly impacts the surrounding environments, which highlights the urgent need for effective removal of oil contaminant from soil. At present, most clean-up efforts for oil spill on soil and shoreline require mechanical and labor intensive methods as they may be a quick and simple solution to remove oil contaminants (Broman et al. 1983). However, there are many disadvantages associated to these methods such as the usage of high pressure washing to displace

oil which may destroy the microbial populations, while the chemical sorbents and dispersants may be harsh to the environment. In addition, these mechanical methods are tedious, time consuming and are only capable of removing the oil contaminants up to a certain extent, leaving behind a large amount of oil adsorbed in the soil. These conventional first response actions are therefore not able to achieve efficient clean-up of oil spills under a short period of time (Lim et al. 2016). Therefore, there is an urgent need to investigate on other environmental-friendly remediation methods to remove oil contaminants in soil.

To date, there is a variety of technologies available for the remediation of oil contaminated soil, ranging from widely applied technologies such as solvent extraction, bioremediation and biosorption to the emerging technologies such as electro-kinetic remediation and sonication. The sorption technologies for oil contaminated soil can be classified into chemical and physicochemical methods. The following sections will discuss about the sorption technology with biomass in detail.

Various biosorbents including cattail fibers, rice husk, cotton fibers, *Azolla filiculoides* biomass, pomelo peel, straw biomass waste, and lignocellulosic biomass of pineapple leaves were used for the oil removal from aqueous solutions. The biomasses of micro- and macroalgae obtained from saline or freshwater have been reported as a suitable and available biosorbent for water decontamination polluted by different micropollutants (Boleydei et al. 2018).

Algae from marine and freshwater environment may have a special relevance for development of low cost sorbents for removal of oil. Interaction of algae with oil is also likely to affect the fate and transport of spilled oil in the environment and the fate of oil in oily wastewater treatment studies involving algae where significant accumulation of oil may occur on biomass.

2.2 Characteristics of biomass

Marine life is defined by its biodiversity, including many species about which relatively little is known. Indeed, there are many exceptional sources of microorganisms, including plants and animals, that exhibit unique features. With respect to biosorption, various living and non-living

biomasses have been reported, such as algal biomass, fungi, and bacteria (Carolin et al. 2017). An ideal biosorbent will possess features such as availability, non-toxicity, high metal-binding capacity, large-scale usability, and regeneration/re-usability (Wang and Chen 2009). The adsorption properties of biosorbents, it should be noted, are dependent on their structural characteristics, e.g., pore distribution, specific surface area, and functional groups (Ramrakhiani et al. 2011). The chemical composition of the biomaterials, meanwhile, is affected by seasonal, temporal, and spatial variations of environmental factors such as water temperature, salinity, light, and nutrient availability (Peña-Rodríguez et al. 2011). The physical and chemical characteristics of biosorbents are important for understanding the metal-binding mechanism on the biomass surface. The characterization of the structure and surface chemistry of the biosorbent is of considerable interest for the development of adsorption and separation processes. Depending on the nature of the biosorbent, a variety of techniques are useful for this purpose, e.g., Fourier Transform Infra-Red (FTIR) spectroscopy, X-ray Photo Electron Spectroscopy (XPS), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Energy Dispersive X-ray (EDX) fluorescence spectrophotometry, and nitrogen sorption. To obtain the structure and complete description of the composition and morphology of the various biosorbents, these methods are commonly used in together (Arief et al. 2008).

(i) Algae

Algae refers to a large and diverse group of unicellular and multicellular organisms distributed in water bodies and terrestrial environments. Algae contain chlorophyll and carry out oxygenic photosynthesis. The colonial forms of algae occur as aggregates of cells in which each cell shares common functions and properties, including the storage products they utilize as well as the structural properties of the cell walls, where the characteristics of a given algal functional group may affect the adsorption process (Pathak et al. 2018; Rangabhashiyam et al. 2014).

Algal biomass is the most widely employed of any biosorbent material. Figure 2-1 shows the SEM images of alga *Ulva lactuca* (Ibrahim et al. 2016), dried *Chlorella vulgaris* (El-Sheekh et al. 2019), *E. intestinalis* biomass (Boleydei et al. 2018). As no treatment is required for algae, it is considered a low-cost biosorbent, and its cell wall characteristics give it a high metal ion-binding capacity (Anastopoulos and Kyzas 2015). Non-living algal biomass has been reported to be more

promising as compared to living algae because of its higher metal ion sorption capacity and rate, and given that it does not require nutrients grown in a medium (Zeraatkar et al. 2016). Different types of algae, such as marine algae, marine red macroalgae, marine brown macroalgae, and freshwater green macroalgae, have been reported as effective biosorbents. Brown algae in particular have been reported to have good biosorption capacity due to the presence of alginates in their cell walls (Bilal et al. 2018).

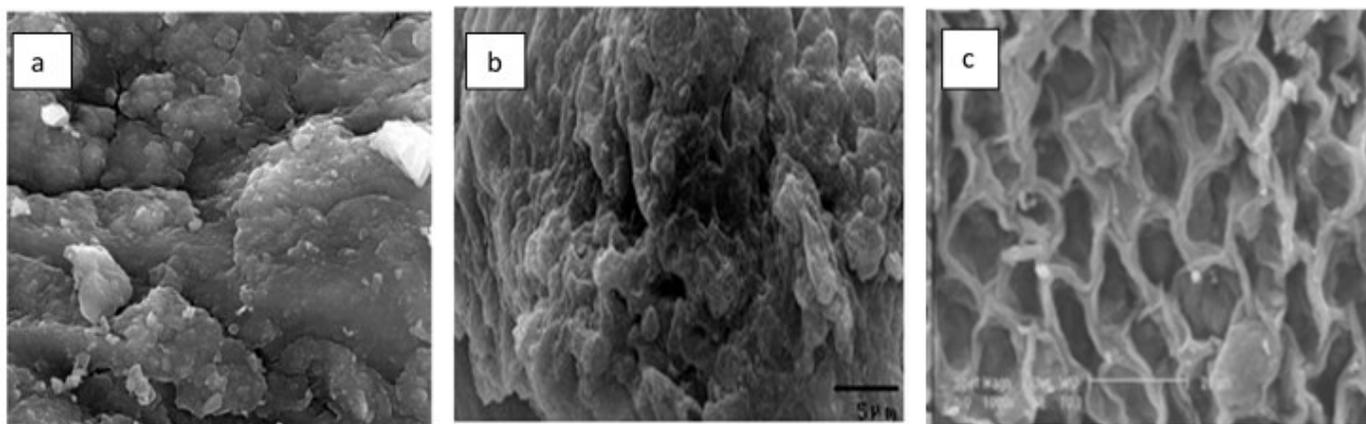


Figure 2-1 SEM images of (a) alga *Ulva lactuca* (Ibrahim et al., 2016), (b) dried *Chlorella vulgaris* (El-Sheekh et al., 2019), (c) *E. intestinalis* biomass (Boleydei et al., 2018)

Algal has many functional groups, such as carboxyl, hydroxyl, and sulphate groups, that are categorized by their complex polysaccharides, protein and, lipid components (Ghadiryfar et al. 2016; Sarı and Tuzen 2008), which in turn are likely to have some bearing on nature of the adsorption process for the various functional groups (Henriques et al. 2017). Since algae do not require nutrients and can be exposed to environments with high toxicity, they have been used widely in various industrial applications in order to reduce hazardous organic and inorganic pollutants and biosorption often makes use of dead biomasses of algae (Crist et al. 1981).

A number of studies have shown that carbohydrates are the main structural component of macroalgae biomasses. The total carbohydrate sources differ according to the species (Hernández-García et al. 2019; Ho et al. 2013; Mata et al. 2010; Nigam and Singh 2011). In a study which was conducted by Flores-Chaparro et al. (2017) they have been reported as accounting for 15.9%, 16.02%, and 11.8% of brown, green, and red macroalgae biomasses, respectively, followed by

hemicellulose in the case of green (13.57%) and red (9.30%) macroalgae samples. For brown macroalgae, meanwhile, protein content has been reported as the second highest component at 13.4%, whereas a lower protein content has been observed in green and red seaweeds (2.8e7.1% dry weight).

The cell walls of algae are complex networks of biopolymers consisting of a skeleton of crystalline and fibrous parts (cellulose, hemicellulose, etc.) and an embedding matrix of specific polysaccharides, proteoglycans, and other molecular components depending on the type of species. Because of the complexity of most macroalgae biopolymers, it is probable that several interactions between pollutants and active sites will passively occur in the system simultaneously (Flores-Chaparro et al. 2017).

(ii) Fungi and crab shells

Fungal cell walls are rigid and contain complex polysaccharides—namely, chitin (which adds structural strength) and glucans—that serve many functions, including providing cell rigidity and shape, metabolism, and ion exchange. Polysaccharides account for 80% to 90% of the fungal cell wall, with proteins, lipids, polyphosphates, and inorganic ions making up the wall-cementing matrix. Two layers have been observed in ultrastructural studies of the fungal cell walls (Arief et al. 2008). Bacteria are a major group of unicellular living organisms belonging to the prokaryotes, which are ubiquitous in soil and water and exist in symbiosis with other organisms. Bacteria have a relatively simple cell structure, lacking nuclei, but possessing cell walls (Seltmann and Holst 2013). The bacterial cell wall provides structural integrity to the cell, but differs from that of all other organisms due to the presence of peptidoglycan (poly-*N*-acetylglucosamine and *N*-acetylmuramic acid). The cell shape and rigidity of each bacterial cell wall are due to the peptidoglycan substance in their structure (Kolenbrander and Ensign 1968). Since the peptidoglycan is porous, it is also has been considered as an barrier to small substrates (Vijayaraghavan and Yun 2008).

Rae et al.(2009) investigated the adsorption performance of adsorbents derived from crab shells for the removal of Hg from acidic solutions and found them to be effective, rapid, and easily

processed. Cai et al. (2019), meanwhile, found the microstructure of crab shell powder to be characterized by a fibrous structure and a loose, unevenly arranged surface. Chitin can be obtained from fungi, insects, lobsters, shrimps and krill, but the most abundant commercial source is the exoskeletons of crabs (No et al. 1989). Chitin is a strong but flexible nitrogen-containing polysaccharide. Chitin and chitosan have been recognized as effective biosorbents for concentrating radionuclides and heavy metals, because their amine and acetamido groups function as nonspecific binding sites (Tsezos 1983). Moreover, they are inexpensive and abundantly available. Chitin, it should be noted, is a natural polysaccharide consisting of (1–4)-2-acetamido-2-deoxy-D-glucose units, while chitosan is its deacetylated derivative. The shell of the crab, *Portunus trituberculatus*, comprises (weight %): calcium carbonate (58), chitin (17), protein (10), moisture (13), and other substances (2). With regard crab shell composition, Lee et al. (2004) showed the calcium carbonate and chitin in crab shell to be effective in the treatment of lead-bearing wastewater, as calcium carbonate forms strong lead-carbonate precipitate while chitin acts as an adsorbent for the precipitate. Figure 2-2 shows surface of heat dried fungus *A. malaysianum* biomass (Majumder et al. 2017), dried flake of bacterial cellulose (*Gluconacetobacter sucrofermentans*) (Atykyan et al. 2020) and crab shell powder (Cai et al. 2019).

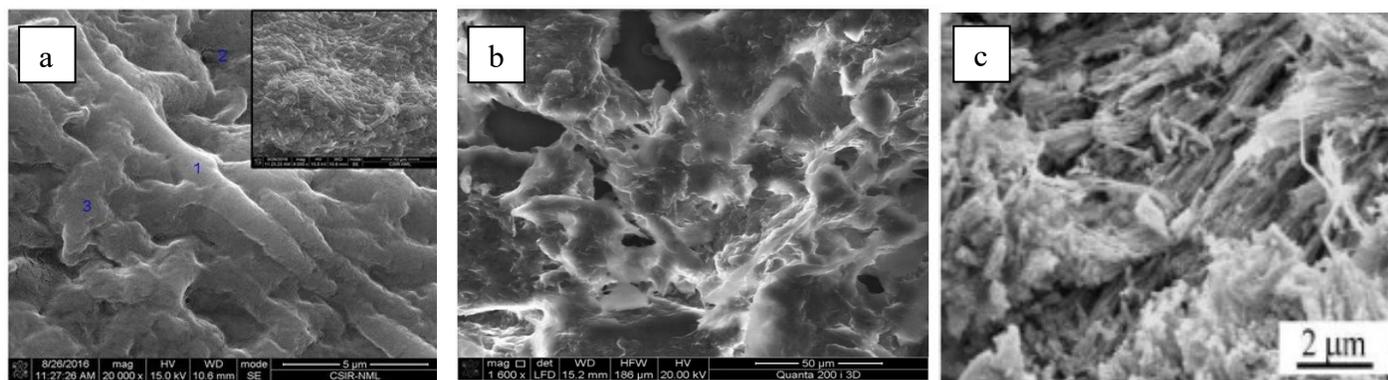


Figure 2-2 SEM images of (a) surface of heat dried fungus *A. malaysianum* biomass (Majumder et al., 2017), (b) dried flake of bacterial cellulose (*Gluconacetobacter sucrofermentans*) (Atykyan et al., 2020), (c) crab shell powder (Cai et al., 2019)

2.3 Removal of petroleum pollutants from water using algae-derived adsorbents

2.3.1 Removal of crude oil using algae-derived adsorbents

Mishra and Mukherji (2012) studied biosorption of diesel and lubricating oil using dead biomass of *Spirulina sp.* and *Scenedesmus abundans*. They observed the rate and extent of sorption in well-mixed batch systems containing oil (0.1% to 2%, v/v) and biomass (0.1%) suspended in water. The sorption of diesel on *Spirulina sp.* was observed to be instantaneous. The Freundlich and Langmuir models, it should be noted, were found to be suitable for diesel sorption on algae but not for lubricating oil. A three-parameter model, meanwhile, was found to be suitable for all experimentally-generated isotherms, yielding maximum biosorption capacities for both diesel and lubricating oil in the range of 12 g/g to 14 g/g for a 12-day period of study. In another study, Boleydei et al. (2018) used an algal biomass of green macroalgae *E. intestinalis* as a biosorbent for decontamination of freshwater and seawater polluted by crude oil and spent oil. They reported that the maximum sorption capacity of both compounds by the biosorbent was higher in seawater than in freshwater. The spent engine oil, with a higher viscosity, showed better sorption efficiency compared to the less viscous crude oil under the given experimental conditions. Moreover, the adsorption data were found to be well fitted to both the pseudo-second-order kinetic model and the Langmuir isotherm model.

Different mechanisms have been reported for the removal of potentially toxic elements through biosorption (Bilal et al. 2018; Chojnacka 2010). Biosorption, as mentioned above, is a passive process that occurs at a faster rate than bioaccumulation. Adsorption, chelation/complexation, and surface precipitation have all been identified as sub-processes of biosorption (Figure 2-3), with the particular mechanism of biosorption being dependent upon the biomass being used for the removal (Flouty and Estephane 2012).

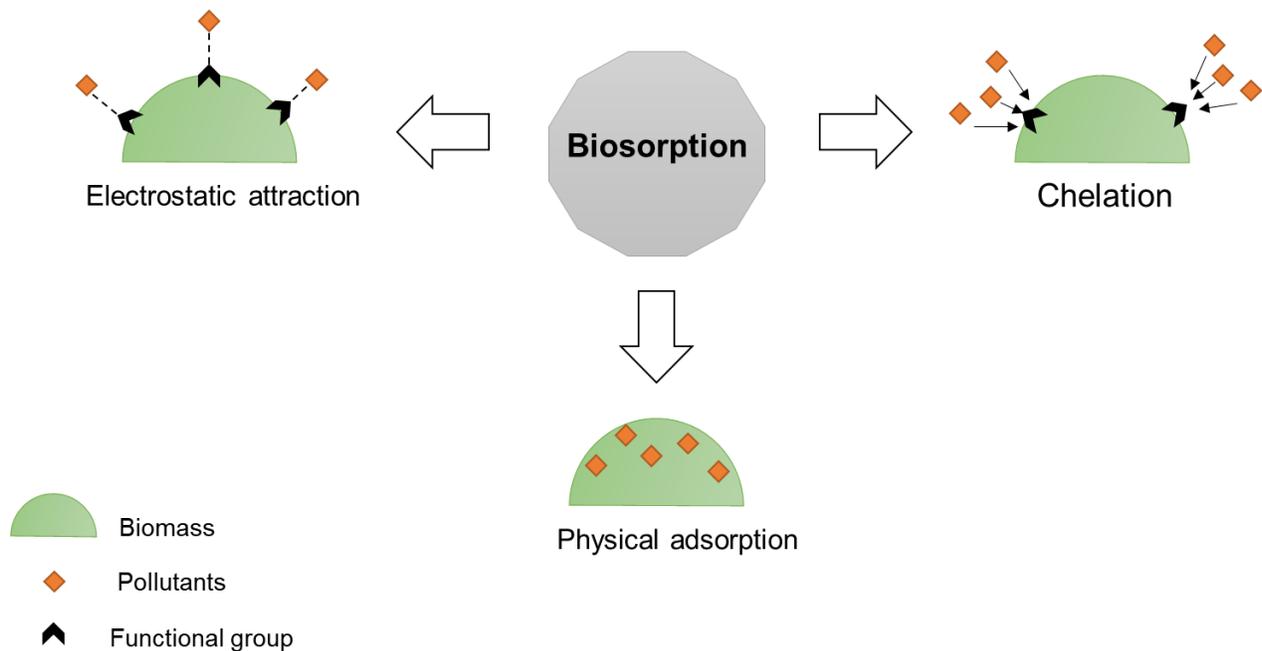


Figure 2-3 Adsorption, chelation/complexation and surface precipitation are different processes involved in biosorption

The principal mechanism involved in biosorption appears to be physico-chemical binding of organic pollutants to the cell surfaces (cell adsorption), and this process does not require any metabolic energy (Avery et al. 1998). The initial contact is characterized by the ion-binding properties of the algal cell walls (Chu et al. 1997; McDonald and Trevors 1988). Algal cell walls offer a host of functional groups, including amino, carboxyl, sulphates, phosphates, and imidazoles associated with polysaccharides alginic acid and proteins for binding various pollutants (Ahmad et al. 2005a; Crist et al. 1981). Algal cell walls, it should be noted, possess an overall negative charge and have receptors capable of binding and attracting cations (Chu et al. 1997; Marbelia et al. 2016). Vandevivere and Kirchman (1993) have noted that the presence of extracellular polymeric substances and the fluidity of the membrane may also be key factors in adsorption. Moreover, Sijm et al. (1998) suggest that algae may also produce and excrete exudates consisting of a significant amount of organic materials, with these exudates binding to heavy metals and organic contaminants on the surface.

2.3.2 Removal of PAHs using algae-derived adsorbents

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants in aquatic ecosystems. PAHs naturally occur in fossil fuels such as coal and petroleum, but they are also formed during the incomplete combustion of organic materials such as coal, diesel, wood, and vegetation (Chekroun et al. 2014). Researchers have reported biosorption of organic micropollutants, such as biosorption of low concentrations of fluoranthene and pyrene on various microalgae (Koelmans et al. 1995; Pathak et al. 2018). Lei et al. (2002) evaluated the efficiency of four microalgal species—*Chlorella vulgaris*, *Scenedesmus platydiscus*, *Scenedesmus quadricauda*, and *Selenastrum capricornutum*—to remove fluoranthene (1.0 mg/L), pyrene (1.0 mg/L), and a mixture of fluoranthene and pyrene (each at a concentration of 0.5 mg/L). Their study found the removal to be algal species-specific and toxicant-dependent. In their 7-day study, *Selenastrum capricornutum* was the species found to be most efficient at PAH removal/transformation (78%), while *C. vulgaris* was the least efficient species in removing and transforming PAHs (48%). All the species under investigation, with the exception of *S. platydiscus*, exhibited higher removal efficiency of fluoranthene than of pyrene. The removal efficiency of fluoranthene and pyrene in a mixture was comparable to or higher than the removal of either compound on its own, suggesting that the presence of one PAH stimulates the removal of the other PAH (Chan et al. 2006; El-Sheekh et al. 2012; Lei et al. 2002; Lei et al. 2007; Safonova et al. 2005).

The batch sorption-kinetics and equilibrium uptake of phenanthrene (PHE), a hydrophobic organic compound (HOC), in aqueous compartments were investigated by Chung et al. (2007), who experimented with dead tissue of the brown seaweed, *Sargassum hemiphyllum*, under various conditions for 24 hr. They found higher shaking rates (50 rpm to 250 rpm) and higher temperatures (15°C to 35 °C) to be associated with higher sorption rates of PHE, although no significant changes were observed with respect to maximum sorption capacities.

Swackhamer and Skoglund (1993) have noted that the cellulose and lipid content in microalgal cell walls may also have an effect on the biosorption processes because of their strong binding affinity to hydrophobic chemicals. Meanwhile, several studies have been conducted to evaluate the contributions of various fractions of algal detritus on adsorptive removal of organic pollutants

in order to better understand the interaction between the detritus and the contaminants. As several studies have noted, fatty acids and proteins are the main substances released from dead algal cells under light (Kumar et al. 2019; Ma et al. 2018; Widrig et al. 1996). These findings indicate that some cellular content in dead algal cells is released from broken membranes into the medium and then induces photocatalytic reactions with pollutants (Luo et al. 2014).

In another study, Zhang et al. (2019a) evaluated the adsorption capacity of three PAH compounds (Phe, BaP, and Naph) and reported that, after the removal of the lipid fractions, the adsorption capacity decreased by as much as 25%. This clearly shows that the lipid fractions play an important role in the adsorption of PAHs. Based on their research, polysaccharide might have less effect on adsorption capacity since after its removal, the adsorption capacity of Phe and BaP have been decreased. In contrary, Polysaccharides removal had positive effect on the adsorption capacity of Naph and increased the adsorption capacity up to 57%. Luo et al. (2014) hypothesized that the high adsorption of PAHs by dead cells may be due to the absence of metabolic protection against the transport of pollutants into the cell, increased permeability of the dead cell membrane, and changes to the surface adsorptive properties of the microalgae cell following its death.

Property of pollutants and the removal of algae substances which result in change in composition and structure of algae could be a logical explanation for adsorption. The diffusion of pollutants with lower hydrophobicity was observed after removal of polysaccharides fraction which result in the formation of larger pores on the detritus. Therefore, increase in adsorption capacity of Naph was observed during this process (Zhang et al. 2019a).

Dead *Selenastrum capricornutum* has been shown to perform well removing phenanthrene, fluoranthene, and pyrene (Chan et al. 2006). One reasonable explanation is that the PHE is removed primarily through a rapid adsorptive removal process, followed by a plant uptake process in the live algae treatments. The principal mechanism involved appears to be physico-chemical adsorption, which is metabolism-independent (Tam et al. 2002). On a related note, Avery et al. (1998) concluded that cell walls provide many potential binding sites for organic and inorganic pollutants, and additional binding sites might become more readily available in dead cells than in

live cells. Dead algal cells also have a higher capacity for PAH adsorption than do live cells. Table 2-1 shows a summary of different types of biosorbents which have been used for pollutant removal.

Gadd et al. (1990) described an experiment in which, in water treated with dead cells, TBT was rapidly removed in the first 5 min (e.g., 85% removal by dead *Chlorella* cells), but then there was no significant difference in TBT removal between 5 min and 3 days of treatment. St-Louis et al. (1997) reported that biosorption by *Pavlova lutheri* occurred almost instantaneously when cells and organotin were mixed (the adsorption of TBT by the exudate coating on the cell wall was in the range of 10% to 20%).

Table 2-1 Use different types of algae-derived adsorbents in the removal of petroleum pollutants

Algae	Compound	Removal rate	Reference
Spirulina sp. and Scenedesmus abundans	Diesel and lubricant oil	Up to 75%	(Mishra and Mukherji 2012)
E. intestinalis	Crude oil and spent oil	80%	(Boleydei et al. 2018)
Sargassum hemiphyllum	Phenanthrene	91.7–98.4%	(Chung et al. 2007)
Ulva prolifera	Benzopyrene, Naphthalene Phenanthrene	ND ¹	(Zhang et al. 2019b)
Chlamydomonas sp., Chlorella miniata, Chlorella vulgaris, Scenedesmus platydiscus, S. quadricauda, S. capricornutum, Synechosystis sp.	Pyrene, Fluoranthene	Up to 78%	(Lei et al. 2002; Lei et al. 2007)
Scenedesmus platydiscus, Scenedesmus quadricauda and C. vulgaris	PAH	ND ¹	(Luo et al. 2014)
Selenastrum capricornutum	Phenanthrene, fluoranthene	Up to 90%	(Chan et al. 2006)
Ulva prolifera	Phenanthrene	91.3%	(Zhang et al. 2017)

¹ Not Determined

2.4 Removal of petroleum pollutants from water using adsorbents derived from fungi, and shells

Microorganisms play an important role in the fate of organic pollutants in the environment, including through biosorption and biodegradation. The use of bacterial cells as a sorbent to remove aqueous phenanthrene and other organic pollutants has been demonstrated in a number of studies (Díaz 2004; Muñoz et al. 2006; Stringfellow and Alvarez-Cohen 1999); however, the PHE removal achieved in these studies varied (42% to 91%). Several studies, meanwhile, have investigated the role of live and heat-killed microbial cells in the adsorption of various pollutants such as NAP, PHE, PYR and crude oil (Rangabhashiyam et al. 2014).

2.4.1 Removal of oil using adsorbents derived from fungus, bacteria and shells

The use of the dead cells of microorganisms such as fungi and bacteria as biosorbent has been studied extensively since fungal biomasses are ubiquitous in aquatic environments (Cheng et al. 2020; Wang et al. 2017; Zhou and Kiff 1991). Xu et al. (2013) conducted a study in which, within the experimental time, the surface adsorption of crude oil using live bacterial cells was found to gradually increase likely due to the fact that crude oil is a complex mixture containing n-alkanes, aromatics, resins, and asphaltenes, some of which adsorb rapidly on dead cells. However, because of the many polar organic pollutants included and the reversibility of the adsorption occurring in the process, the adsorbed crude oil was released from the dead cells.

Devi et al. (2012) demonstrated that natural biodegradable chitosan of low molecular weight derived from crab shell can remove most of the colloidal and suspended organic matter in vegetable oil mill effluent. It was observed that the removal efficiency of adsorbent was optimum at a pH of 4. They also found that chitosan performs well as an adsorbent due to its efficiency in treating effluent, noting the minimal adsorbent dosage required for the treatment process. Cai et al. (2019) conducted a study to investigate the adsorption kinetics of crab shell-derived biochar with mesoporous structures, finding that it possesses a high capacity to adsorb diesel oil (about 93.9 mg/g). As they noted, this is due to the high specific surface area ($2.441 \text{ m}^2/\text{g}$), excellent pore

volume (1.682 m³/g), and unique surface characteristics present in functional groups such as hydroxyl (–OH–) and carboxyl (–COO–).

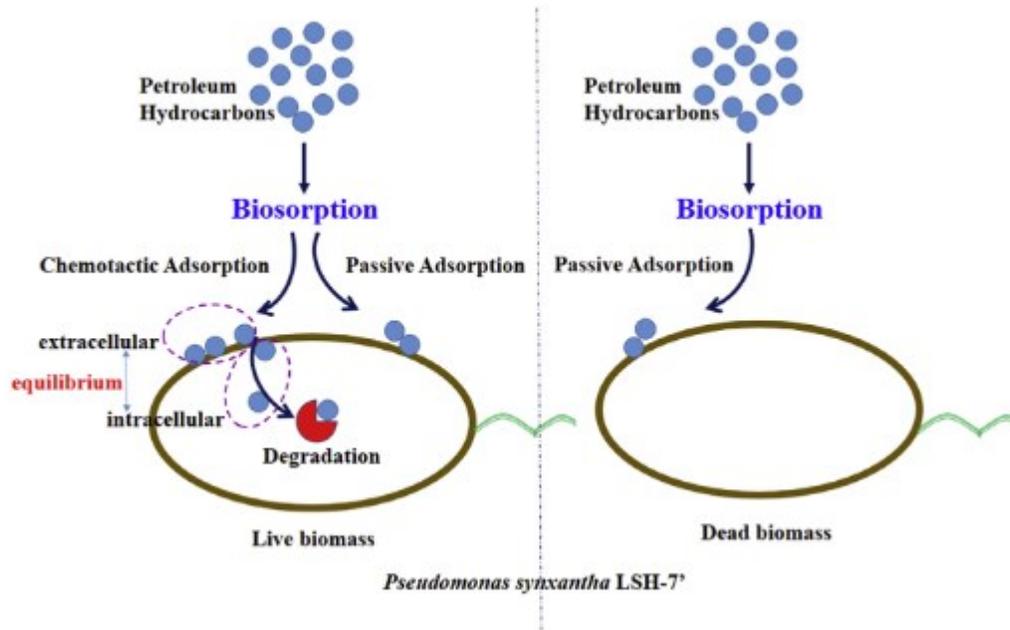


Figure 2-4 Sketch picture of bacterial chemotactic biosorption (Meng et al., 2019)

2.4.2 Removal of PAHs using adsorbents derived from fungus, bacteria and shells

Numerous studies have been conducted investigating the transport role in the surface adsorption of different pollutants by dead and live microbial consortia (Tsezos and Bell 1989). As many researchers have noted, the bacterial cell wall acts as the primary component for interacting with pollutants such as metal ions or dyes, where they are sorbed on the surface or within the cell wall (Doyle et al. 1980; Kim et al. 2020; Simelane et al. 2018). Kumar et al. (2008) noted that dead fungal biomasses possess a high capacity for biosorption of toxic pollutants such as heavy metals. Moreover, studies on the biosorption of organic compounds with fungal biomass have shown that enhanced phenolic removal is achieved more efficiently using dead fungal biomass than using a live biosorbent (Rao and Viraraghavan 2002).

Xu et al. (2009) explored the adsorption of different substrates by a heat-killed microbial consortium, investigating in particular the role of four different heat-killed bacteria, *Pseudomonas sp.*, *Bacillus sp.*, *Ochrobactrum sp.*, and *Pseudomonas sp.*, in the adsorption over time of petroleum hydrocarbons. The adsorption of PAH compounds achieved by the heat-killed microbial consortium was found to be constant. Due to its high hydrophobicity, PYR was found to be adsorbed more efficiently than PHE and NAP, and this finding is consistent with the results of (Ding et al. 2013). The stability of the surface adsorption of NAP, PHE, PYR, and crude oil by the microbial consortium followed a decreasing order as follows: NAP > PHE = PYR > crude oil. NAP, PHE, and PYR were found to be more easily adsorbed by the microbial consortium than was crude oil up to the 1-minute mark of experimentation, and it was inferred that the process of surface adsorption by a microbial consortium is a rapid process (Xu et al. 2013).

Raghukumar et al. (2006) studied the removal of polycyclic aromatic hydrocarbons from an aqueous culture medium using marine fungus *NIOCC # 312*. They attributed the removal disappearance of phenanthrene from the aqueous culture medium to its instant adsorption to the fungal biomass, asserting that heat-killed fungal biomass could be used for the efficient adsorption of PAHs from contaminated sites. Chen et al. (2010) reported the effect of fungal biomass on the biosorption of polycyclic aromatic hydrocarbons in aqueous solutions. They found that the bio-adsorption of heat-killed bodies was related to the carbon-normalized partition coefficients and suggested that sorption of organic pollutants in biomass may involve simultaneous surface sorption, partitioning, and chemical reactions.

Both Chen et al. (2010) and Ding et al. (2013) have attributed these phenomena to the bio-adsorption behavior of microbial cells with respect to both PAHs and alkane, this, in turn, being governed by the distributional effects of the sorption–desorption process being reversible. The possible mechanisms reported by Chen et al. (2010) that the bioadsorption of heat-killed bodies was relevant with the carbon-normalized partition coefficients.

2.5 Major factors which can impact the process

Many factors, such as the type and nature of the biomass or derived product and freely suspended cells, can affect biosorption. Physical and chemical treatments such as boiling, drying, autoclaving, and mechanical disruption will all also affect the binding properties. For instance, chemical treatments such as alkali treatment often improve biosorption capacity, this being especially evident in some fungal systems because of deacetylation of chitin to form chitosan-glucan complexes with higher metal affinities (Malik et al. 2002; Wang and Chen 2006).

Several parameters such as biomass age, nutrition of the biomass and growth medium can also have effects on biosorption since they change the cell size and wall composition. Another important parameters for each cells and particles are the ratio of surface area to volume area and the available surface area of immobilized biofilms. Also, increasing biomass concentration may have effect on biosorption capacity because it clearly reduces the sorption per unit weight (Chojnacka 2010; de Rome and Gadd 1987). Other physico- chemical factors such as pH , temperature and ion strength are also have an effect on biosorption (Wong-Ng et al. 2018).

(i) pH

Biosorption in most systems including bacteria, algae and fungi has seen to be pH- dependent. pH variation can influence the transfer of oil from sand to the aqueous phase in the presence of microalgal biomass. pH has complete effect on several factors including competition of sorbate ions and solution chemistry of the adsorbate. It also influences the activity of the functional groups in the biosorbent. Variations in pH also affect the effectiveness, as the hydrogen ion itself is a tough competing adsorbate (Puranik et al. 1999). Highly alkaline conditions (a pH of 10.0 or higher) have been shown to lead to a decline in biosorption efficiency (Lim et al. 2016). Changes in zeta potentials of oil and algae at different pH levels also play an important role in oil removal (Lim et al. 2016). Several mechanisms have been proposed for sorption of oil on algae including once the absorption/partitioning of oil on to organic matter and another is adsorption on to the surface of algae due to specific interactions. Adsorption which is nonlinear sorption isotherms is a slower phenomenon compared to the absorption/partitioning of HOCs which can be explained by linear isotherm (Al-Shamrani et al. 2002).

(ii) Temperature

The effects on biosorption of another environmental parameter, temperature, have also been investigated in a number of studies. Temperature has a vital effect on biosorption process, as increase or decrease in temperature can influence the extent of biosorption (An and Huang 2012). By increasing the surface activity and kinetic energy due to temperature increasing, the removal of pollutants enhances. Contrarily, this may also damage the physical structure of the biosorbent (Park et al. 2010; Pathak et al. 2018). In another study, Rajaković-Ognjanović et al. (2008) found increasing temperature to have a positive effect on oil sorption on algae, as the oil viscosity decreases with increasing temperature. From the microscopic perspective, elevated temperatures are also favorable for enhanced movement of adsorbate molecules from adsorbent. Although increasing temperature is typically favorable to pollutant desorption, though, it should be noted that there could be an increase in the porosity and total pore volume of the biomass adsorbent. In addition, an increase in temperature could result in the swelling of the adsorbent's internal structure and increased penetration of larger oil molecules (Choi and Moreau 1993; Toyoda et al. 2000).

(iii) Ionic strength

Chung et al. (2007) identified a correlation between increased sorption and increased ionic strength. However, Mishra and Mukherji (2012) noted that the trend in q_e variation with ionic strength was significantly affected by the type of algae. They observed, for instance, that the sorption of both lubricating oil and diesel on *Spirulina* sp. was highest at the lowest ionic strength (background electrolyte, NaNO_3 , concentration of 0.01 M), decreased for NaNO_3 concentrations of up to 0.1 M, then increased significantly at 0.5 M and subsequently decreased again as the NaNO_3 concentration was increased to 1 M. The sorption of lubricating oil and diesel on *S. abundans*, meanwhile, continuously decreased up to 0.5 M and 0.1 M NaNO_3 concentrations, respectively, and subsequently remained invariant as the NaNO_3 concentration was increased up to 1 M. Chung et al. (2007) reported that maximum sorption of phenanthrene by brown seaweed was attained at low ionic strength, at neutral pH, and at temperatures ranging from 30 °C to 35 °C. In their study, the expected trend for dissolved organic solute, i.e., an increase in

sorption with an increase in ionic strength, was not observed for oil sorption on algae. The trend in specific oil/diesel uptake variation with ionic strength, meanwhile, was found to be significantly affected by the type of algae. Beolchini et al. (2006) showed the negative effect of ionic strength on biosorption performance: for instance, the highest determined value for copper-specific uptake was approximately 60 mg/g at a pH of 6 and approximately 15 mg/g at a pH of 4.

CHAPTER 3. MATERIALS AND METHOD

3.1 Materials

The microalgal biomass used in this study was derived from *C. vulgaris* and the corresponding powder was obtained from the Organic Traditions (Montreal, Canada). Standard washed and ignited sand (30-40 mesh) was purchased from MilliporeSigma (Oakville, Canada). Crude oil was obtained from Hibernia oil field located in the southeast of St. John's, Newfoundland, Canada. The properties of sand and oil are shown in Table 3-1 and Table 3-2, respectively. Other chemicals were purchased from Sigma-Aldrich (Oakville, Canada).

Table 3-1 Sand specification used in this research

Specification	Value
Particle size form and distribution	Granular, 30 to 40 mesh
Formula	SiO ₂
Color	Beige
Molecular Weight (g. mol ⁻¹)	60.08
Density (g. cm ³)	2.622 at 20°C
Soluble substances (in HCl)	0.2% max

Table 3-2 Typical Characterization of crude oil

Characterization	Value
API gravity	37.1
Density at 20 °C (g. mL ⁻¹)	0.8370
Dynamic Viscosity at 20°C (cP)	33
Pour point (°C)	6
Hydrocarbon groups (weight%)	Saturates (79), aromatics (15), Resins (4), Asphaltenes (3)

3.2 Experimental procedure

Batch tests were used to investigate the effect of microalgal biomass on the oil behavior in a sand-water system. A certain amount sand and crude oil was mixed to achieve the initial concentration of 4 g oil/kg sand. The contaminated sand was placed in fume hood for weathering of 7 days. In each test, 0.5 g of contaminated sand was added into the 20-mL vial. 15 mL of solution containing various amount of dry weight microalgal biomass was then added into the vial, followed by shaking on a reciprocal shaker at 300 rpm for 24 hours. After that, the aqueous phase in the vial was removed and 15 mL of hexane was added into the vial followed by shaking for 24 hours to extract the remaining oil from sand. Different biomass dose, pH, temperature, and salinity levels were used in these tests. Solution pH was adjusted with 1 M NaOH or HCl. The vials with water in the absence of microalgal biomass were used as the controls. All the tests were conducted in triplicate and the mean values were used in data analysis.

3.3 Factorial design

To further evaluate the effects of environmental conditions on oil behavior, a 2⁴ full factorial design was employed. Four factors including microalgal biomass dose, solution pH, temperature, and salinity were considered with the response of remaining oil (mg kg⁻¹). All factors were studied at two levels. The low and high levels were coded as -1 and +1, respectively Table 3-3. 48 runs of experiments were conducted and the details are shown in Table 3-4. Minitab 15 statistical software (Minitab Inc., State College, USA) was used to design experiments and analyze the experimental data.

Table 3-3 Coded and uncoded values of the factors.

Symbol	Factor	Units	Level	
			Low (-1)	High (+1)
A	A	ppm	500	5000
B	pH		5	9
C	Temperature	°C	10	20
D	Salinity	%	1	3.5

Table 3-4 Coded levels and corresponding values for factorial design matrix.

Run Number	Coded levels				A	B	C	D
	A	B	C	D	Biomass Dose (ppm)	pH	Temperature °C	Salinity (%)
1	-1	-1	-1	-1	500	5	10	1
2	-1	1	-1	-1	500	9	10	1
3	-1	-1	-1	1	500	5	10	3.5
4	-1	1	-1	1	500	9	10	3.5
5	1	-1	-1	-1	5000	5	10	1
6	-1	1	1	-1	500	9	10	1
7	1	-1	-1	1	5000	5	10	3.5
8	1	1	-1	1	5000	9	10	3.5
9	-1	-1	1	-1	500	5	20	1
10	-1	1	1	-1	500	9	20	1
11	-1	-1	1	1	500	5	20	3.5
12	-1	1	1	1	500	9	20	3.5
13	1	-1	1	-1	5000	5	20	1
14	1	1	1	-1	5000	9	20	1
15	1	-1	1	1	5000	5	20	3.5
16	1	1	1	1	5000	9	20	3.5
17	-1	-1	-1	-1	500	5	10	1
18	-1	1	-1	-1	500	9	10	1
19	-1	-1	-1	1	500	5	10	3.5
20	-1	1	-1	1	500	9	10	3.5
21	1	-1	-1	-1	5000	5	10	1
22	-1	1	1	-1	500	9	10	1
23	1	-1	-1	1	5000	5	10	3.5
24	1	1	-1	1	5000	9	10	3.5

25	-1	-1	1	-1	500	5	20	1
26	-1	1	1	-1	500	9	20	1
27	-1	-1	1	1	500	5	20	3.5
28	-1	1	1	1	500	9	20	3.5
29	1	-1	1	-1	5000	5	20	1
30	1	1	1	-1	5000	9	20	1
31	1	-1	1	1	5000	5	20	3.5
32	1	1	1	1	5000	9	20	3.5
33	-1	-1	-1	-1	500	5	10	1
34	-1	1	-1	-1	500	9	10	1
35	-1	-1	-1	1	500	5	10	3.5
36	-1	1	-1	1	500	9	10	3.5
37	1	-1	-1	-1	5000	5	10	1
38	-1	1	1	-1	500	9	10	1
39	1	-1	-1	1	5000	5	10	3.5
40	1	1	-1	1	5000	9	10	3.5
41	-1	-1	1	-1	500	5	20	1
42	-1	1	1	-1	500	9	20	1
43	-1	-1	1	1	500	5	20	3.5
44	-1	1	1	1	500	9	20	3.5
45	1	-1	1	-1	5000	5	20	1
46	1	1	1	-1	5000	9	20	1
47	1	-1	1	1	5000	5	20	3.5
48	1	1	1	1	5000	9	20	3.5

3.4 Analytical methods

The oil concentration in hexane was measured by a Thermo Scientific Evolution 201 UV-Visible spectrophotometer (MA, USA) with wavelength of 220 nm. A Jenway 3310 pH meter (Jenway Ltd., UK) was used to determine the pH of solution. The IR spectra of crude oil, algae, and algae with crude oil were recorded in triplicate using a Tensor 27 FTIR–ATR Spectrometer (Bruker, USA).

Fourier-transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time. FTIR can be used in all applications where a dispersive spectrometer was used in the past. In addition, the improved sensitivity and speed have opened up new areas of application. Spectra can be measured in situations where very little energy reaches the detector and scan rates can exceed 50 spectra a second. Fourier transform infrared spectroscopy is used in geology, chemistry, materials and biology research fields. Fourier transform infrared (FTIR) spectroscopy has been widely used in the analysis of oil (Deivakumari et al. 2020; Xin et al. 2019b). It can generate unique spectral signatures related to the absorption pattern of crude oil with the major bands (Hadjoudja et al. 2010). Asemani and Rabbani (2020) studied FTIR spectroscopy characterization of crude oil extracted asphaltenes. In their study, four oil samples were collected from different oil fields and Asphaltene extraction from crude oil samples was analyzed using FTIR. In another study which was conducted by Riley et al. (2016), ten different oil samples from various geographical regions were collected and the fractions of the crude and heavy fuel oils were analysed on a Perkin Elmer Spectrum 400 FTIR/FT-NIR Spectrometer (Perkin Elmer, Australia). Kök et al. (2017) also conducted crude oil characterization using FTIR during their study on four different origin crude oil samples. The use of Fourier transform infrared (FTIR) spectroscopy in algal biomass analysis has also been reported in monitoring biochemical changes (Ibrahim et al. 2010). Xin et al. (2018) conducted a research about the toxicity of triclosan to green microalga *chlorococcum sp.* In their study, FTIR was used to determine the characterization of algae.

The functional groups involved in oil sorption can be identified via the determination of the chemical structure of sorbents before and after oil sorption (Boleydei et al. 2018; Cheu et al. 2016). Sorption has been correlated with the type of functional groups present on the algal cell wall as determined through FTIR studies Mishra and Mukherji (2012). In the present study, to better understand the interaction and identify the functional groups involved during the contact between oil and algae, the oil was mixed with microalgal powder and FTIR analysis was conducted to determine the chemical structure of microalgal biomass (*C-vulgaris*) before and after sorption of oil. ATR-FTIR spectroscopy generated unique spectral signatures related to the absorption pattern of crude oil with the major bands.

Samples were placed on to the diamond radiation area of the ATR device. Each spectrum was an average of 128 scans in the 4000 to 400 cm^{-1} spectral range, using transmission mode for crude oil and reflection mode for algae samples with a resolution of 4 cm^{-1} . The spectra were collected and processed using OPUS 7.2 software (Bruker, USA). FTIR spectromicroscopy was conducted to image single microalgal cell with or without crude oil on beamline 01B1–01(MidIR) at CLS. A Bruker Vertex 70v interferometer coupled to a Hyperion 3000 IR confocal microscope with a 15 \times objective was applied to acquire the images using a Globar (Xin et al. 2019a). Spectral images were acquired using reflection mode for algae samples with a resolution of 4 cm^{-1} . Each spectrum was an average of 128 scans, using a 64 \times 64 pixel mid-infrared FPA detector. Three repeats for each sample were conducted using OPUS 7.2 software and the images were generated using CytoSpec 2.00.01 software (Cytpspeg Inc., USA). The surface morphology of algae biomass was measured by Quanta 400 scanning electron microscope (FEI, USA). Meanwhile, the elemental constituents were also analyzed by SEM-EDS. The particle size of microalgal biomass was determined by Mastersizer 3000 laser diffraction system equipped with Hydro EV exchangeable volume wet sample dispersion unit (Malvern Instruments, USA).

3.5 Quality assurance/quality control

The quality assurance/quality control program was followed to ensure the accuracy and reliability of the collected data. All batch tests were conducted in triplicate and average values were reported

(relative standard deviation less than 5%). Blank tests were run and corrections were applied if necessary. All containers used in this study has been previously cleaned with the particular washing liquid for laboratory purposes, triply rinsed with distilled water and oven dried. For each experimental run, oil contaminated sand and deionized water was used without addition of any algal powder. The corresponding results confirmed that the initial concentration of solution including contaminated sand remained unchanged during the experiment. It indicated that the reduction of oil concentration in contaminated sand during adsorption tests was only related to the presence of algal biomass. Statistical calculations and analyses were performed with the use of the Minitab (Minitab Inc., USA) statistical software package.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Characterization of microalgal biomass interacted with oil

Chlorella is a group of eukaryotic green microalgae with a high capacity for photosynthesis and growth (Nigam and Singh 2011). These microalgae have simple life cycles and metabolic pathways similar to higher plants. Therefore, they have been employed as indicator organisms for research on the mechanisms of photosynthesis and assimilation of carbon dioxide (Nabavi and Silva 2018). Figure 4-1(A) shows the SEM images of *C. vulgaris* biomass. *C. vulgaris* is a spherical microscopic cell with 2–10 μm diameter and it shares many structural elements similar to plants. The rigidity preserves the integrity of the cell and is basically a protection against invaders and harsh environment. It may vary according to each growth phase (Safi et al. 2014). As a natural biomass, the algae cells are mainly composed of many organic matters. According to the EDS results shown in Figure 4-1(B), the amounts of C, O, P and S of microalgal biomass were 35.9, 40.6, 0.3 and 0.2%, respectively. Hao et al. (Hao et al. 2017) conducted the elemental analysis for this strain and got the similar results of 46.67% C, 6.92% H, and 37.97% O. Some mineral elements such Si, Na, Cu and Ca were also observed in this microalgal biomass. Figure 4-1(C) depicts the particle size distribution of algal biomass. It can be seen that 10% of the microalgal particles had a diameter less than 30.2 μm and 50% of the particles had a diameter less than 64.8 μm . Almost 90% of the microalgal particles were with the diameter less than 121 μm . Microalgae particle size is smaller than macroalgae. Such a small size of microalgae let them move easily with current and waves, and they can also enter on the void space of beaches.

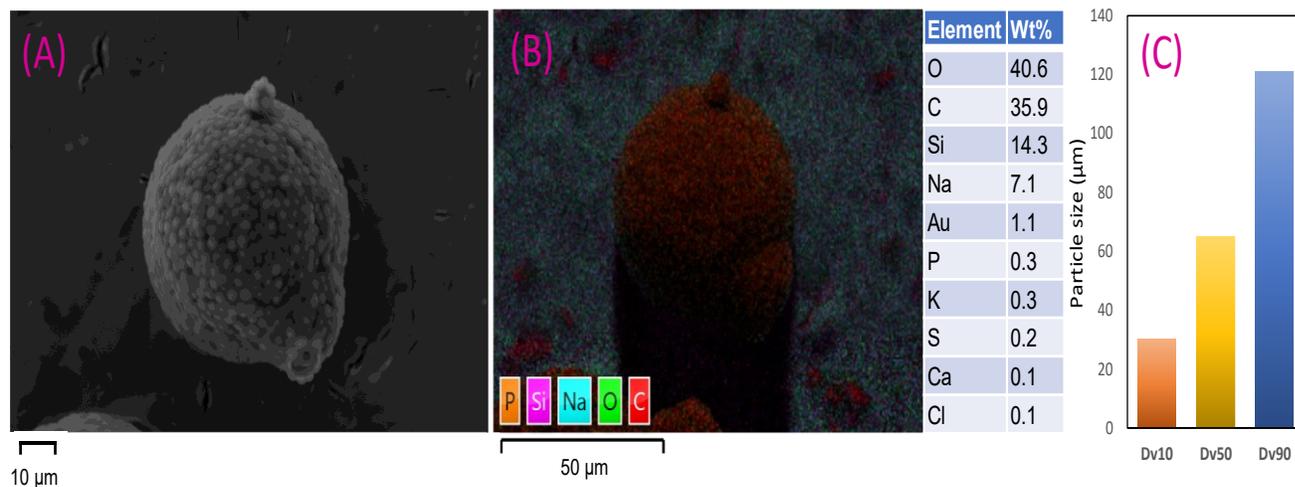


Figure 4-1 (A) SEM image of *C. vulgaris* cell, (B) Energy Dispersive X-Ray Spectroscopy (EDS) of *C. vulgaris* cell, (C) Particle size analysis of *C. vulgaris* biomass

As microalgal biomass in solution contacted with oil-contaminated sand, there would be an interaction between oil and microalgal biomass. To better understand such interaction and identify the functional groups involved during the contact between oil and algae, the oil was mixed with microalgal powder and FTIR analysis was conducted to determine the chemical structure of microalgal biomass (*C-vulgaris*) before and after sorption of oil. ATR-FTIR spectroscopy generated unique spectral signatures related to the absorption pattern of crude oil with the major bands shown in Table 4-1. Comparing to algae, ATR-FTIR spectra of algae with crude oil presented spectral signatures of crude oil (Figure 4-2(A)). For algae with crude oil, the infrared spectra at 2952, 2920 and 2853 cm^{-1} were more intensive than that of algae, resulting from the contribution of the stretching aliphatic bands (ν -(CH_3+CH_2)) from crude oil. Similarly, there were also more intensive bands at 1459 and 1377 cm^{-1} , corresponding to the deformation bands of methyl and methylene groups from crude oil. A new band appeared at 1708 cm^{-1} was attributed to carbonyl and/or carboxyl groups (ν -($\text{C}=\text{O}$)), observed in crude oil spectra but not in algae spectra. In the region 800-900 cm^{-1} , two bands occurred at 810 and 870 cm^{-1} which could be assigned to the out-of-plane deformation vibration of C-H bond (γ (CHar)) of aromatic ring from crude oil. Comparing with algae, the occurrence of a new peak and the increases of many other band peaks in the infrared

spectra of algae with crude oil was attributed to the sorption of crude oil onto algae. The most distinguishable spectra peaks include aliphatic hydrocarbons, carbonyls and aromatics. FTIR spectromicroscopic imaging can also provide a straightforward evidence that crude oil could be sorbed by algae. As shown in (Figure 4-2 (B2-F2)), single mircoalgal cell with oil had more intensive signal in the spatial distribution of main functional groups, such as aliphatic hydrocarbons, carbonyls and aromatics from crude oil, compared with mircoalgal cell without oil (B1 - F1). The band centered at 1708 cm^{-1} was integrated and newly occurred in algae sample with crude oil. This was attributed to the sorption of crude oil, which is consistent with infrared spectra results. The integrated groups were distributed evenly around the cell, indicating the distribution of sorbed crude oil was homogeneous. Since algae powder used in this study didn't include living algae, it is likely that crude oil was unable to enter into cells but could be sorbed around mircoalgal cell surface. Moreover, the intensified color presented the sorption amount of crude oil was substantial. Therefore, there could be an interaction between algae and oil once they contacted.

Table 4-1 Wavenumber (cm^{-1}) and assignment of the major bands of ATR-FTIR spectra of crude

Wavenumber (cm^{-1})	Vibrational mode assignment and main contribution
2952, 2920, 2853	stretching aliphatic bands (ν (CH_3+CH_2))
1708	carbonyl and/or carboxyl groups (ν -($\text{C}=\text{O}$))
1459	deformation bands of methyl and methylene groups (δ - (CH_3+CH_2)).
1377	deformation bands of methyl (1365 cm^{-1})
870, 810	out-of plane deformation vibration of aromatic C—H bond

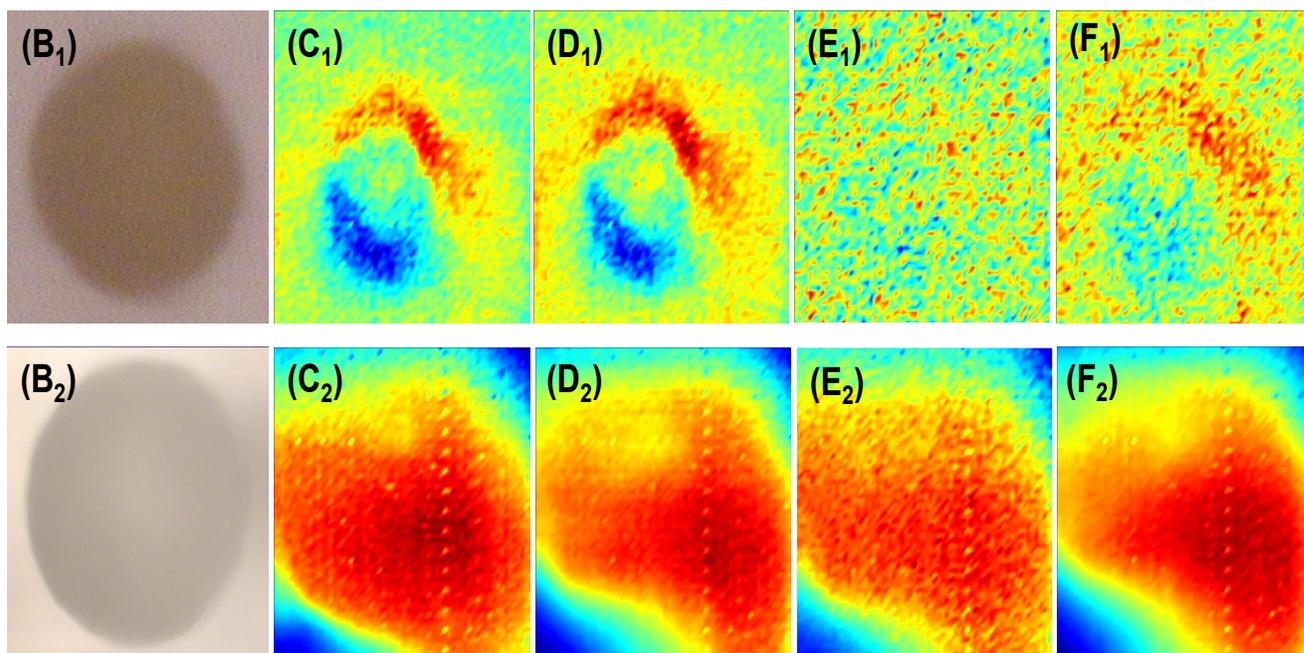
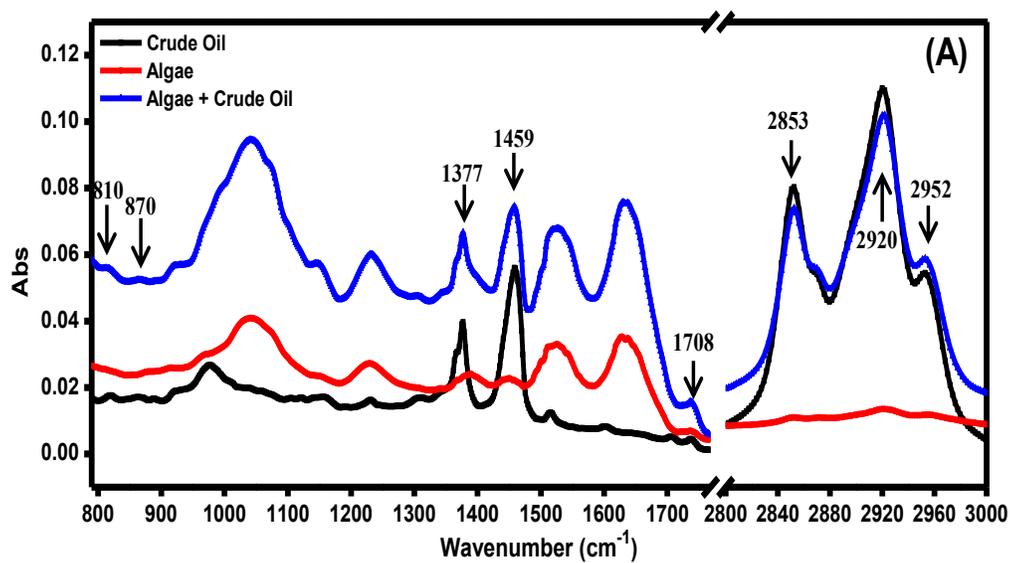


Figure 4-2 ATR-FTIR spectra for crude oil, algae, and crude oil * algae (A). Microscopic imaging (B₁, B₂) and FTIR spectromicroscopic imaging for samples of algae, and crude oil * algae at 3001-2765 cm^{-1} (C₁, C₂), 1490 – 1326 cm^{-1} (D₁, D₂), 1721 – 1694 cm^{-1} (E₁, E₂)

4.2 Effect of microalgal biomass dose on oil behavior

The impact of microalgal biomass dose ranging from 0.01 to 8 g L⁻¹ on oiled sand was studied with the pH of 7, temperature of 20 °C and salinity of 0%. The results are shown in Figure 4-3. It can be found that when the dose of microalgal biomass increased, the amount of remaining oil on sand decreased. It took about 5 g L⁻¹ of microalgal powder to reduce residual oil concentration to 1065 mg kg⁻¹. This decrease of remaining oil on sand tended to be stable when the microalgal biomass dose was higher than 5 g L⁻¹. It was reported that the adsorption of oil increased as biomass concentration changed from 0.01 to 8 g L⁻¹ (Boleydei et al. 2018). In this sand-water system, the microalgal biomass could be bound with oil. The surface area of microalgal powder was high due to the small particle size (Chojnacka et al. 2005). There were also many micropores on algal powder (Ibrahim et al. 2010). The increase of microalgal biomass dose provided more functional groups such as aliphatic bands in algal cells and active sites for the binding of oil. The biomass powder could also act as the bridge for binding oil in liquid (Ahmad et al. 2005b). Nwokoma and Anene (2010) found also reported that algal particles less than 150 µm presented the highest removal of oil from water with short time reaching equilibrium. In addition, there was an equilibrium between the oil in water and the oil on sand surface. Once the oil droplets in water were adsorbed on microalgal powder, it would facilitate the shifting of equilibrium and resulting more desorbed oil from sand. The shallow and nutrient-rich water in the coastal area provides an ideal place for the growth of algae. The algal biomass amount can even reach a very high level when there is an algae bloom. Such impact of algal biomass amount should be taken into consideration for the evaluation of oil behaviors on oiled shoreline.

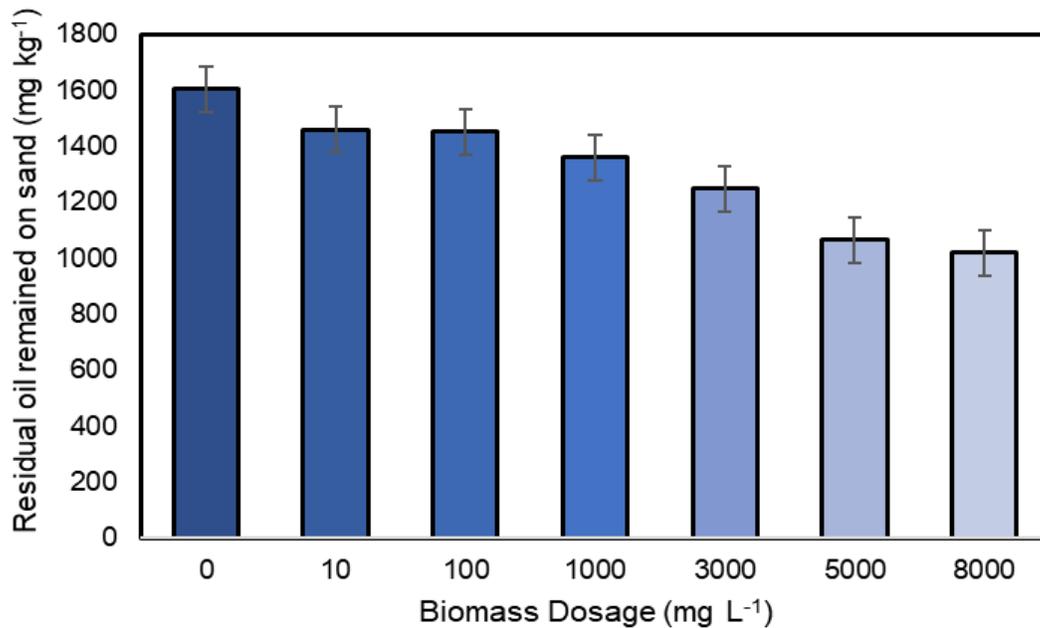


Figure 4-3 The effect of biomass dose on the oil behavior in a sand-water system.

4.3 Effect of pH on oil behavior

Although, The pH of solution is an important parameter which can affect the pollutant transport and binding (Ibrahim et al. 2010; Zhao et al. 2019). pH in seawater and shoreline sediments may vary considerably in the environment (Cleary et al. 2018). In order to explore the effect of pH on oil behavior in the presence of microalgal biomass, the tests were conducted in the pH range of 4-10 with the microalgal biomass dose of 5000 ppm, temperature of 20 °C and salinity of 0%, and the results are shown in Figure 4-4. The remaining oil decreased with increasing solution pH to 7 and an increase of remaining oil was observed when the pH was further increased above 7. The results indicate that pH variation could influence the transfer of oil from sand to aqueous phase in the presence of microalgal biomass. The changes in zeta potentials of sand, oil and algae at different pH levels played an important role in this process. It was reported that both sand particles and oil droplets were with negative charge regardless of pH levels (Lim et al. 2016). As pH increased from 4 to 7, there could be increased differential surface charge between sand particles and oil droplets (Lim et al. 2016), resulting the higher repulsion between sand and oil and less oil

remained in sand. The binding of oil onto biomass including algae has been observed (Boleydei et al. 2018; Mishra and Mukherji 2012). The oil removed from sand could be adsorbed by microalgal biomass, with the relatively stable surface charge was in this pH range. Therefore, the overall conditions were favorable for the increased oil removal and less oil remained on sand. As pH further increased above 7, an increase of remained oil on sand was observed. On the one hand, the oil could become less negative at high pH (Al-Shamrani et al. 2002). That would be favorable for the binding between oil and sand. On the other hand, the algae surface could also become more negative at high pH (Malik et al. 2002). Such conditions would thus facilitate the oil binding to sand and less adsorption of oil on microalgal biomass in this sand-water system.

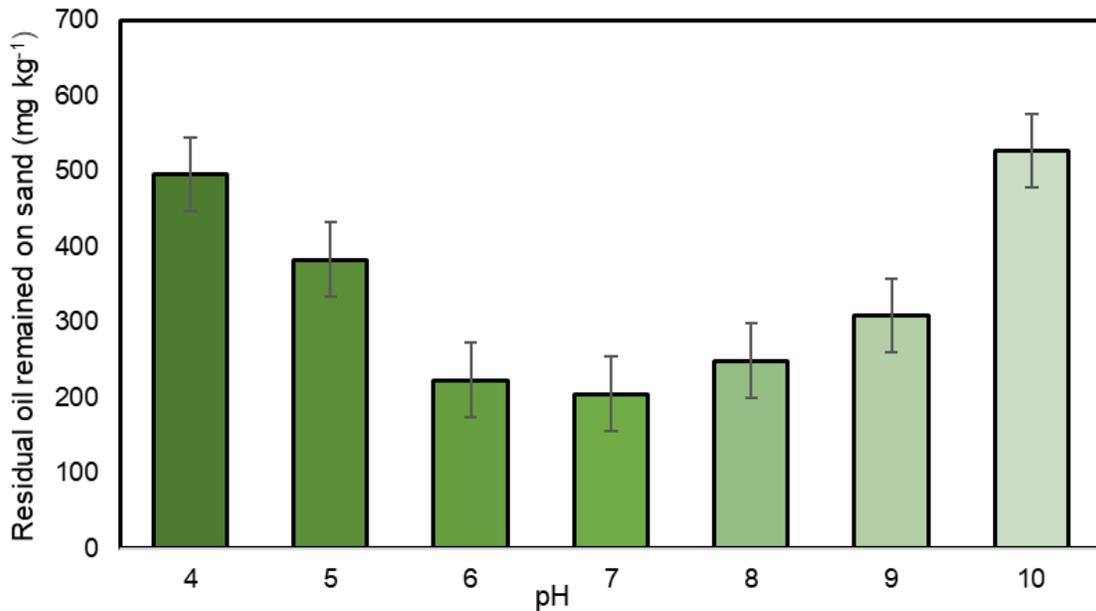


Figure 4-4 The effect of pH on the oil behavior in a sand-water system

4.4 Effect of temperature on oil behavior

The temperatures of near-surface sea and shoreline vary with latitude in response to incoming solar radiation and outgoing longwave radiation. The temperature can range from 30 °C at the tropical region to -1 °C (30.2 °F) at the arctic region. The effect of temperature on oil behavior in the presence of algal by mircoalgal biomass was studied at different temperatures from 5 to 30 °C with the microalgal biomass dose of 5000 ppm, pH of 7 and salinity of 0%. As shown in Figure 4-5, the oil remained on sand in the presence of microalgal biomass decreased as temperature increased. The remaining oil on sand was 636.9 mg kg⁻¹ at 5 °C, while it decreased to 222.9 mg kg⁻¹ at 25 °C. Temperature is as an important parameter that can often affect pollutant behavior (An and Huang 2012). The oil viscosity would decrease with the increasing temperature. The lower viscosity at high temperature could facilitate the oil removal from oiled sand to liquid phase. From the microscopic view, the elevated temperatures are also favorable for enhanced movement of adsorbate molecules from adsorbent (Rajaković-Ognjanović et al. 2008). Once oil left from sand into solution, oil was further bound with microalgal biomass. Although increasing temperature was usually favorable to the pollutant desorption, there could be an increase in the porosity and total pore volume of biomass adsorbent. In addition, the increase in temperature could result in the swelling of adsorbent internal structure and increased penetration of larger oil molecules. Similar results were also observed in previous studies about the adsorption of oil on nonwovens cotton and exfoliated graphite (Choi and Moreau 1993; Toyoda et al. 2000) Therefore, the overall effects of these processes let to the decreased remaining oil as temperature increased.

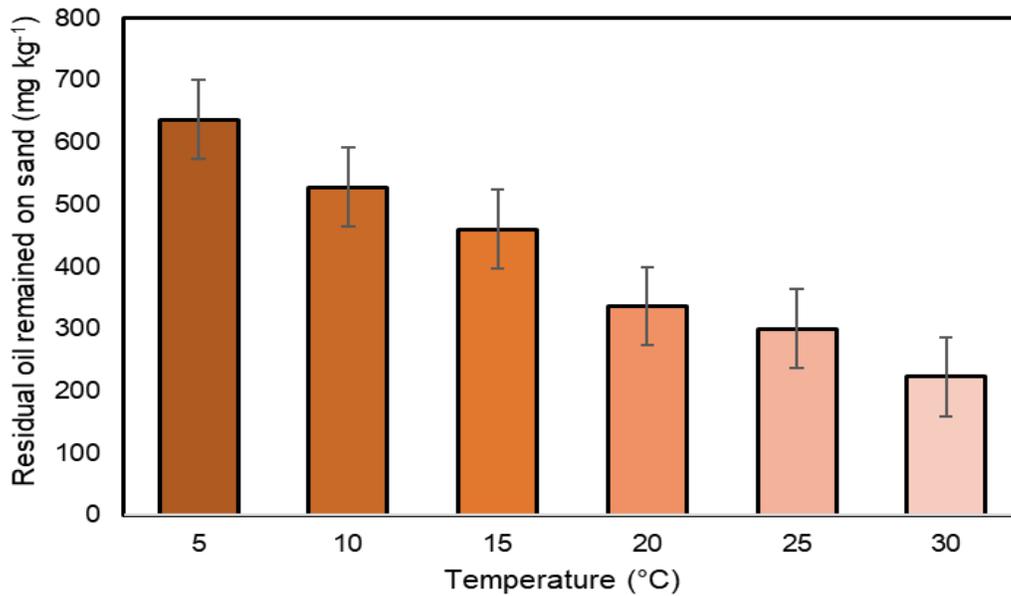


Figure 4-5 The effect of temperature on oil behavior in a sand-water system

4.5 Effect of salinity on oil behavior

The average salinity of the sea water typically varies from 3.2% to 3.7%. It may be less than 3% in polar regions. The departure from a mean salinity of approximately 3.5% is usually caused by processes at Earth's surface that locally add or remove fresh water. In the region of the ocean receiving the fresh water such as estuaries, the salinity may be lowered by dilution. The effect of salinity on oil removal in the presence of microalgal biomass was investigated in this study and four different salinity levels up to 4.5 % were evaluated with the microalgal biomass dose of 5000 ppm, pH of 7, and temperature of 20 °C. As shown in Figure 4-6, the changing salinity can affect the oil behavior in this system. Increasing the salinity level from 0 to 3.5% resulted in decreasing remained oil in sand from 205 to 82 mg kg⁻¹ correspondingly. The algae cells and oil droplets is usually negatively charged. Adding sea salt including positive ion like Na⁺ could affected the surface charge of biomass. As the salinity increased, there would be a compressed electric double layer, making it easier for particles to approach each other and form oil particle aggregates (Le Floch et al. 2002). It was reported there was no noticeable change of surface charge of oil droplets as salt concentration increased (Liu et al. 2007). However, increasing salinity could increase the

surface charge of biomass powder (Lin et al. 2003). As the salinity increased, the favorable oil adsorption by algae would result in the decreased oil remained on sand. When the salinity further changed to 4.5%, the residual oil on sand increased to 100.59 mg kg⁻¹. The solubility of oil decreased due to the salting-out effect at high salinity, which could facilitate the sorption of oil on sand. In addition, the high salinity was correlated with the smaller algae surface area (REED et al. 1980). That would limit the combination of oil and microalgal biomass and thus left more oil on sand and in water.

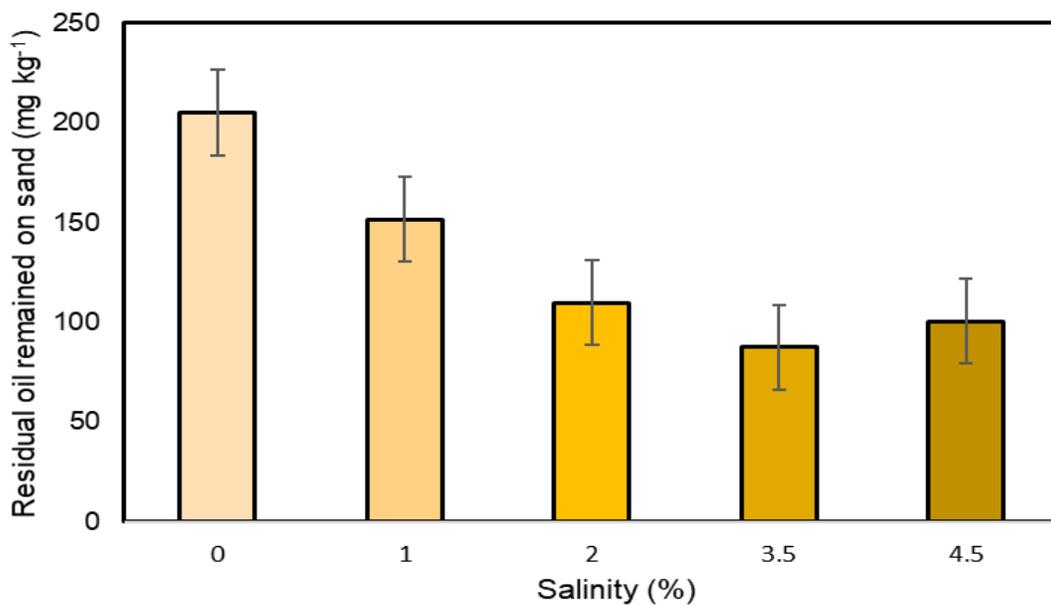


Figure 4-6 The effect salinity on oil behavior in a sand-water system

4.6 Factorial analysis of environmental conditions and its implications

A full factorial design with two levels has been conducted to determine the contribution of these influencing factors. In statistics, a full factorial experiment is an experiment whose design consists of two or more factors, each with discrete possible values or "levels", and whose experimental units take on all possible combinations of these levels across all such factors. A full factorial design may also be called a fully crossed design. Such an experiment allows the investigator to study the

effect of each factor on the response variable, as well as the effects of interactions between factors on the response variable. Figure 4-8(A-D) shows the main plot for four factors with two levels for each factor, which can meet the requirement of factorial design. The detailed contribution of each factor has also been studied with more different levels in previous sections of this manuscript.

In the routine experiments, only one factor can be studied each time with all the other variables being constant, which can hardly reveal the potential interactions among factors on response variables (Zhao et al. 2016). Factorial design can reveal not only the effects of individual parameters but also their relative importance in given process. The interactive effects of two or more variables can also be revealed (Xin et al. 2016). The detected potential interactions among experimental factors will be meaningful for providing a deep insight into the complicated mechanisms or processes under the influences of factors at different levels. In full factorial designs, an experimental run is performed at every combination of factor levels (Shen et al. 2017). Specifically, the two-level factorial design has a sample size that is a power of two, 2^k , where k is the number of factors He et al. (2018). Thus, in our study, a 2^4 factorial design was determined to study the significant factors and their interactions for the oil behavior, in which four variables were assayed at two levels. The factorial analysis of variance (ANOVA) was performed to identify the main effects of factors and their interactions in oil removal. The effects of the factors would be positive on response if the linear effects are positive. Reversely, the effects of the factor would be negative on response if the linear effects are negative. The statistical significance of individual and interaction effects of the experimental factors was checked by the Fisher F-test, and model terms were evaluated by the p-value with 95% confidence level (He et al. 2018; Huang et al. 2018). Overall, the factorial design is an effective tool to evaluate the significant impacts of environmental factors and their interactions, which is why we used the method in this study.

The study of interactive effects of various factors can help better under the environmental process (Liu et al. 2020). The statistical analysis results are shown in Table 4-2. Figure 4-7 shows the Pareto chart which presented the main and interaction effects in the process. The effects that are above the reference line are statistically significant at 95% confidence level. The results showed that the microalgal biomass dose had the most significant effect on the removal of oil. A positive sign of the effect indicated that a high factor setting (+1) would result in a higher oil removal. An

increase in microalgal biomass dose from 500 ppm to 5000 ppm resulted in the decreased residual oil sand to as low as 34%. The higher biomass concentration may lead to less remained oil due to the more functional groups and active sites toward oil. Since adsorption is a surface-related phenomenon and microalgal powder has the internal porosity, they create adsorption forces as well as an adsorption surface area. Also, due to the small particle size of microalgal powder, surface area is high and may help more on adsorption process. The effects of factors on oil behaviors increased in the following order: biomass dose > temperature > salinity > pH.

Table 4-2 Estimated effects and coefficients for residual oil remained on sand

Term	Effect	Regression Coefficient	Standardized effect	P-value
A-Biomass Dose	-3.566	-1.783	-4.93	<0.001
B-pH	1.211	0.606	1.68	0.102
C-Temperature	-2.095	-1.048	-2.90	0.006
D-Salinity	-2.0	-1.0	-2.77	0.009
AB	1.181	0.59	1.63	0.111
BD	1.134	0.567	1.57	0.125
AD	-1.113	-0.557	-1.54	0.132
BC	0.856	0.428	1.18	0.244

Pareto Chart of the Standardized Effects

(response is Residual oil(mg/Kg), Alpha = .05)

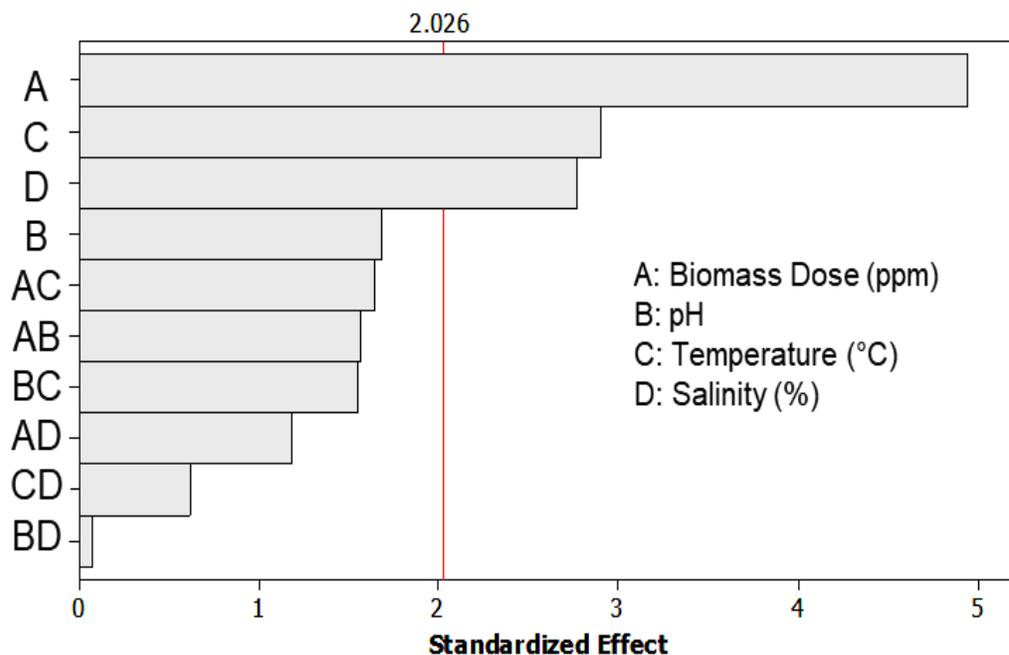


Figure 4-7 Pareto chart for standardized effects for Residual oil remained on sand

The main effects of individual factors on oil remained on sand are shown in Figure 4-8 (A-D). The effect of microalgal biomass dose has been characterized by a greater degree of departure from the overall mean, indicating factor A-biomass dose has a large positive effect on oil removal. The increase of biomass dose resulted in the decrease of remained oil on sand. In the oiled shoreline, the natural oil removal process can be re-evaluated if the high algae amount exists. Temperature and salinity showed a slight and moderate positive effect on oil removal. An increase in temperature and salinity would lead to the decrease of remained oil on sand.

The significant two-factor interaction effects on oil behaviors are shown in Figure 4-8(E-J). It is noted that although the main effects of individual factors B-pH and D-salinity on oil behavior were not as significant as other factors, their interaction effects with other factors were significant. When it referred to two-factor interactions for oil removal process, the interactive effects of DB and AB were significant. As seen in Figure 4-8(E), the effect of low-level pH on oil remained on sand was noticeable with increasing of salinity. When the pH of solution was at low level, the oil which was remained on sand was only about 525 mg kg⁻¹ under the salinity level of 1%. However,

approximately 75 mg kg^{-1} difference in remained oil was observed between the two levels of salinity concentrations in low pH. In low salinity, the amount of oil remained on sand was approximately the same under high and low level of pH. It was also noted that the interactive effects of pH and microalgal biomass dose were significant (Figure 4-8(F)), while the other interactions among other factors were not significant. Compared with the results from the single-factor tests, there was a more noticeable difference for the remained oil at different pH levels with changing microalgal biomass dose. As shown in Figure 4-8(G), there was a slight difference between oil remained on sand under different pH levels at low level temperature. At high temperature, however, a noticeable difference of the oil remained on sand was observed at high and low levels of pH. Fig. 4-6(H) shows the interactive effect of salinity and microalgal biomass dose on oil remained on sand. It can be seen that the lowest remained oil on sand could be obtained at high salinity level with increasing microalgal biomass dose. The oil remained on sand could also change under various salinity levels at low and high temperature (Figure 4-8(I)). Less oil remained on sand was obtained under high salinity level at high temperature. Figure 4-8(J) shows the interaction of microalgal biomass dose and temperature on oil remained on sand. There was a big difference between oil remained on sand at low and high dose of microalgal biomass at low and high levels of temperature. At high level of microalgal biomass dose, oil remained on sand decreased noticeably with increasing temperature.

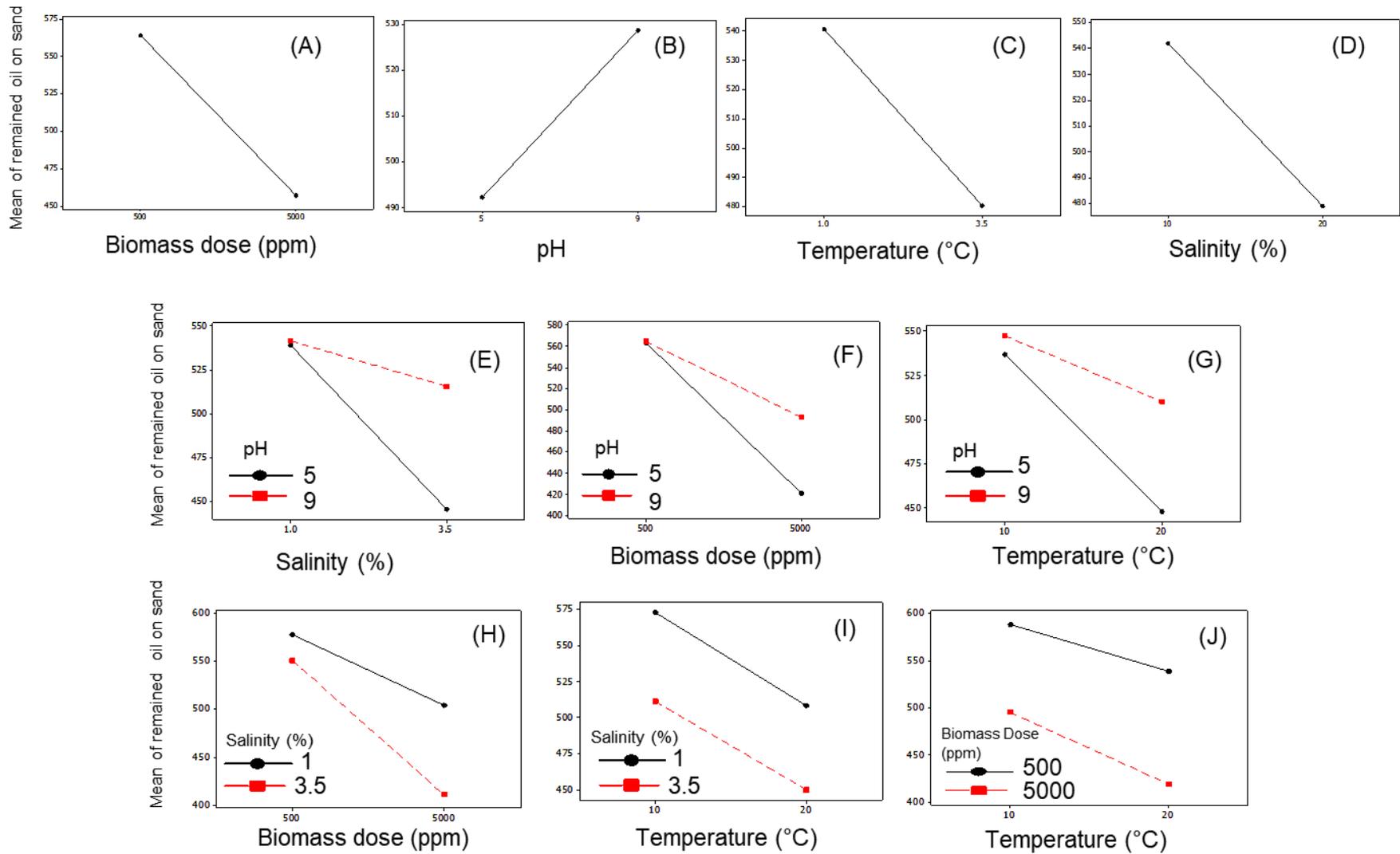


Figure 4-8 (A-D) Main plot for four factors, (E-J) interaction effect plot for each two factors.

CHAPTER 5. CONCLUSIONS

5.1 Summary

In this study, the effects of microalgal biomass on the oil behavior in a sand-water system has been investigated. The microalgal biomass was characterized and the interaction between microalgae and oil was analyzed through FTIR. The effects of different conditions including microalgal biomass dose, pH, temperature and salinity on the oil behavior were investigated. A two-level factorial analysis was also used to further explore the interactions of these conditions. The microalgal biomass was found to be the most influential parameter for the residual crude oil on sand. Higher microalgal biomass dose resulted in less residual oil on sand. The remaining oil decreased with increasing solution pH from 4 to 7, and an increase of remaining oil was observed when the pH was further increased above 7. In addition, temperature and salinity could affect the removal of crude oil in the presence of microalgal biomass. The behaviors of oil in this sand-water system could be influenced by the solution chemistry. The results of this study indicate that the presence of algae in the oil-contaminated shoreline can be considered in the comprehensive evaluation of spill risk and prediction of oil fate. Further study is also required to investigate the impact of oil characteristics and obtain more theoretical foundation for oil mobility under various shoreline environmental conditions.

5.2 Recommendation for future study

The potential of using biomass as biosorbent for organic pollutant sorption has been studied. pH, temperature and ion strength are important factors affecting the rate of biosorption. In different literature, it has been found that most of the research has been limited to single PAH compound biosorption. Biosorption of other organic pollutants such as crude oil and mixed PAH are still in blooming stage, with huge unexplored aspects. This aspect needs to be addressed as in nature more than one compound could be present that may affect the biosorption and degradation of one on the other. pH of biosorption medium is the most important parameter influencing the biosorption capacity. Components of pyruvate dehydrogenase complex have been reported in various studies suggesting key role of the enzyme in degradation of PAH compounds. However, more elaborative research is required to understand other enzymes and mechanism responsible in the degradation of PAHs.

The studies on water soluble hydrocarbon fraction are still limited, with a knowledge gap relative to environmental effects on organic pollutants removal. Biosorption is a ubiquitous property of living or dead biomass and derived products, and is undoubtedly an important process in the environment, and in several conventional waste treatment processes. It has been proposed as a cheap and effective biotechnology for many years, yet has had extremely limited industrial exploitation to date.

In the field of biosorption, microorganisms such as algae, bacteria, fungi have gained immense attention for their abilities to accumulate organic pollutants in their cell structures. However, research studies exploring the utilization of microalgae in biosorption of organic contaminants such as Crude oil and PAHs are limited. Common suggestions for future research directions include identification of better and more selective biosorbents, more development of biosorption models and identification of biosorption mechanisms, and further assessments of market size, and costs of development. The potential of biosorption can be further improved by evaluating the biomass removal capacity for the most water-soluble hydrocarbons (benzene and toluene) under controlled laboratory conditions to increase our knowledge of its use for remediation purposes.

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