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Dynamic Behaviour of the Floating Water Droplet with Hydrocarbon Degrading Bacteria on Oil Surfaces

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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Date: 16.12.2015

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Publications

The following publications were produced in conjunction with the material used in this thesis.

Journal Article

F. Asadi, C.M. Phan, E. Obanijesu, *Dynamic behaviour of a floating water droplet with hydrocarbon-degrading bacteria*, Journal of Environmental Chemical Engineering, (2015). Volume (3), pages (2311-2316), Elsevier.

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Conference Presentation

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Abstract

Oil spillage has been an environmental problem globally. Different physical, chemical, and biological methods are used to contain and decompose the oil spillages, but they are unable to remove oil completely. Recently, floatation of a water droplet on oil surface has been proposed as a new method to decompose oil. This study investigated the influence of hydrocarbon-degrading bacteria on the floating water droplet, which aims to explore a safer treatment method for oil spillages.

An aqueous solution was prepared by mixing a bacterial mixture, Sodium Dodecyl Sulphate (SDS), Enzyme solution and NaCl, and then deposited on the surface of paraffin oil. A commercially available bacterial mixture, containing up to 60 species, was used to achieve high biodegradability. Sodium chloride was employed to mimic seawater. Furthermore, SDS which is the anionic surfactant was used to achieve floatability of a water droplet on oil surface. The shape of the droplet was monitored with a digital camera, with 3X magnification. Consequently, the droplet profile was detected by MATLAB software to find contact angles and volume of a droplet. Furthermore, to verify this new method, different surfactants, medium and environment were used.

The result showed that floatable volume of the bacteria-containing droplet was smaller than the previously reported droplet, which was stabilized by surfactant only. However, the water droplet remained floating sufficiently for bio-decomposition of the oil. The bacterial activities have distinguished influence on the floating behaviour. First, the biofilm was formed at the air/water interface and noticeably reduced the evaporation. Second, the water was completely consumed within 40 minutes.

The overall findings showed that different type of surfactants has the similar effects on the bacterial activities and water droplet shape. Furthermore, it was confirmed that medium plays

an important role in floatation of the water droplet and consequently oil decomposition since it can greatly affect the bacterial activities. Finally, the study on diesel oil revealed the importance of water/air interfacial phenomena in floatation of water droplet since the floatation time of water droplet on diesel oil was less than on paraffin oil.

The results provide important new insights for removing oil spills efficiently by an economical and environmentally friendly method. Transportation of dispersants and equipment in to the location oil spillage takes few days so this method is applied in few hours to complement the current method by degrading the oil layers and prevent from oil contamination of vast area. This method is expected to apply quickly because all the materials can be stored on-site. Only small amount of material is required in addition to seawater. The material cost of the droplet method is estimated to be around 8 times cheaper than currently used methods. The new method can be combined with current practices to reduce the ecological impact of oil spills.

Nomenclature

ΔP	Local hydrostatic pressure in the bulk phase	KPa
R	Radius	mm
J	Surface curvature	Dimensionless
h_3	Height of the “holm” on the water droplet	m
V	Volume of water droplet	mm^3
I	Evaporation rate	gr/min.mm
m	Mass of droplet	gr
t	Time	min
e	Evaporation rate	gr/cm^2. s
A	Area	Cm^2
V_b	Volume of bubble	µl
V_1	Volume of air/water section	µl
V_2	Volume of water/oil section	µl

Greek letters

Γ	Surface tension	mN m^{-1}
γ_{oa}	Interfacial tension between oil/ air interface	mN m^{-1}
γ_{ow}	Interfacial tension between oil/ water interface	mN m^{-1}
γ_{wa}	Interfacial tension between water/ air interface	mN m^{-1}
θ	Tangent angle	o
θ_1	Contact angle between water/air interface	o
θ_2	Contact angle between oil/air interface	o
θ_3	Contact angle between oil/air interface	o
P	Density	Kg m^{-3}
ρ_a	Density of air	Kg m^{-3}
ρ_o	Density of oil	Kg m^{-3}
ρ_w	Density of water	Kg m^{-3}

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Chapter 1: Introduction

1.1. Background to the study

Oil contamination has been considered as an acute environmental problem internationally. Due to transportation accidents, lack of maintenance, rupture of oil pipelines and seepage of crude oil from the floor of sea, oils are accidently released into the environment (1-3). It is estimated that about 6 million tons of crude oil is discharged into the environment per year (4). For example, the oil spillage in Prince William Sound in 1989 was estimated 41.6 million litres (5). Another catastrophic oil spillage was in the Gulf of Mexico in 2010 which 795 million litres oil entered into the environment (6, 7).

1.2. Crude oil and environmental impacts

Crude oil mainly comprises of hydrocarbon substances. The state properties of these hydrocarbons depend on their molecular weight (8). Different sources of crude oil may consist of different compounds such as n-alkanes, branched alkanes, aromatics, cycloalkanes, isoprenoids, asphaltene (9). The extent of oil contamination in the environment depends on the amount and properties of released crude oil. For example, paraffin wax that composed of heavy hydrocarbon components is considered non degradable (4, 10). In addition, asphaltenes containing hydrocarbons and a few percent of heteroatoms such as sulfur, nitrogen, oxygen, vanadium and nickel, are resistant to natural degradation and dispersion (5, 10).

Oil in a water body results in drastic biological damages. The severity of these impacts depends on types of ecosystem, oil component structures and the volume of spilled oil (5, 11). Since oil can scatter over a very large surface area, it may result in adverse damages to animals, ecosystems and human health, as in the case of Gulf of Mexico (2010).

1.3. Effect of natural processes on spilled oil

Once being released into the environment, oils may experience a number of natural processes such as spreading, evaporation, dissolution, dispersion, formation of emulsions, biodegradation, accumulation and sedimentation to the seafloor. These processes can change oil's physical and chemical properties. These changes may decrease oil toxicity and may accelerate recovery of the contaminated area (12, 13). However, aromatic compounds, which are toxic and potentially carcinogenetic, cannot be degraded simply under natural condition. Consequently, they may persist for a long time (5, 13, 14) and serverly damage the ecological systems.

1.4. Current methods to remove oil spillages

There are different methods can remove the oil spillages. None of these methods are perfect. The physical methods that can reduce oil spreading but cannot eliminate it. Chemical treatments such as dispersants which reduce the interfacial tension between water and oil (7, 15) may be toxic for the environment (9). Furthermore, solvents in chemical treatment methods can be evaporated quickly and surfactants may be subjected to biological degradation (15). Therefore, a huge amount of dispersants is required for oil removal. Ineffectiveness of physical skimming and chemical dispersants in the Gulf of Mexico has been reported (16). Another treatment method is bioremediation which is environmentally friendly (14) and could decrease the oil concentration (5). However, an effective bioremediation requires sufficient contact between oil and oil-degrading bacteria, nutrients and oxygen availability (5, 9, 17, 18).

In summary, current methods are unable to effectively address oil spillage problem. Thus, innovative technologies are required to remove oil contamination efficiently.

1.5. New phenomenon

In a previous study (19), floatability of a water droplet on oil surface was confirmed in a very narrow condition. It has been discovered that water droplet with volume up to 170 µl can float on vegetable oil, but mineral oils such as hexane could not support the water droplet. Furthermore, the stability of water droplet on oil surface can be increased by surfactants (20). In this study, the phenomenon is expanded and combined with bioremediation to increase the oil removal. Specifically, the effect of bacteria in floatability of a water droplet on oil surface is verified. The potential of flotation of a water droplet on oil surface in addressing the limitation factors of bioremediation will be verified.

Ultimately, this project intends to use environmentally friendly substances instead of chemical materials which may have some adverse effects on the environment. In addition, a floatable water droplet can increase the available surface area and also address the lack of nutrients for bacterial growth.

1.6. Study objectives

The aim of this study is to determine the floatability of water containing bacteria on oil surface. This research pursues the following objectives:

- Determination of dynamic behaviour of a floating water droplet with hydrocarbon-degrading bacteria
- Variation of medium, surfactants and oily environment for verification of effectivity of water droplet on oil surfaces
- Quantifying the effect of bacterial activities and bioprocess on droplet behaviour by optical monitoring and analysing the droplet shape.

1.7. Organization of Thesis

Chapter 2 presents a literature review of physical, chemical and bioremediation methods. The effectiveness and limitations of these methods will be evaluated. Biodegradation mechanisms in oil decomposition and also different microbial approach for oil removal are emphasized. Moreover, a review of flotation of a water droplet on oil surface and different factors affecting the stability of water droplet are presented. These include the reported effects of biological activities on the oil/water and air/water interfaces.

The methodology and materials used for experimental works are presented in chapter 3. Moreover, image processing and all theoretical analysis for contact radius, contact angle and volume of water droplet floating on oil surface will be presented.

In Chapter 4, the experimental observations of the shape of water droplet containing bacteria are presented. Numerical results obtaining from the fitting procedure will be presented and justified.

In Chapter 5, different materials are used to verify the efffectiveness of this new method. MLA medium and its effects on bacteria are showed. In addition, the influence of different surfactants in biological activities and droplet shape is verified. Finally, this method in the decomposition of diesel oil, which is considered as priority pollutants, is examined.

Chapter 6 concludes the study with recommendations for future work.

Chapter 2: Literature Review

Widespread use of crude oil and the refined products can increase the possibility of oil spills in the environment. For the verification of oil spillage impacts and the fate of released oil in the ecosystem, crude oil and its different classifications are briefly presented. Also, the available methods for addressing of oil spillages are reviewed. Subsequently, bioremediation as a most effective method for decomposition of oil contaminants is disccused. Finally, the possible influences of these factors on the floatability of water droplets are presented.

2.1. Oil products

Crude oil is a mixture of hydrocarbons resulting from the anaerobic conversion of dead organisms such as plants and animals. Over many years, these materials changed to petroleum products under high temperature and pressure and beneath of the Earth's surface (21). The gas formation was under the high temperature while the oil formation was under the low temperature (22). Petroleum is produced from prehistoric zooplankton and algae under anoxic condition.

2.1.1. Components of crude oil

The composition of crudes from different sources varies massively. Crude oil consists of different elements which are carbon, hydrogen, sulfur, nitrogen, oxygen, minerals, and salt. All of these elements are joined with different chemical structures, which are listed hereafter.

2.1.1.1. Saturated aliphatic hydrocarbons, alkanes or normal paraffin

Alkanes or saturated hydrocarbons with the general chemical formula C_nH_{2n+2} consist of hydrogen and carbon atoms with only single bonds. Alkanes ranging from C1 to C40 or more carbon have been identified in crude oil, and also they comprise 20 to 50% of crude oil (23).

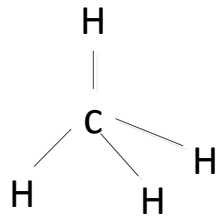


Figure 2-1. Methane (no double or triple bonds between carbon atoms)

2.1.1.2. Saturated cyclic hydrocarbons or cycloparaffins or naphthenes

In saturated cyclic hydrocarbons, carbon and hydrogen are linked together with the only single atomic bonds which are likened to alkanes. However, cycloalkanes are distinguished by the presence of rings of carbon atoms. They have saturated closed rings sequences. The Naphthene or cycloalkanes with a single ring have a formula of C_nH_{2n} and with more than two attached rings has a formula of C_nH_{2n-2} (24). They are plentiful in crude oil and can be found up to 30 to 40 wt % (25).

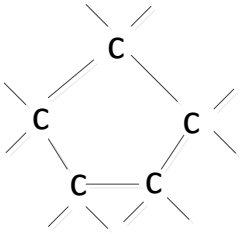


Figure 2-2. Cyclopenthane (a ring of five carbon atoms joined by single bonds)

2.1.1.3. Aromatics

Cyclic and polyunsaturated aromatics are a large class of unsaturated chemical compounds known by rings with the formula of C_nH_{2-6}. Aromatic hydrocarbons comprise carbon atoms are joined by double bonds. For instance, benzene, toluene, ethylbenzene and xylenes and Polycyclic Aromatic Hydrocarbons (PAHs) are aromatic compounds with alternating single

and double bonds and different ring numbers. PAH which are omnipresent in a variety of environmental sections contains two or more rings (11). These kinds of hydrocarbons are carcinogenic and broadly scattered because of incomplete combustion of organic substances, emission sources and automobile exhaust (26).

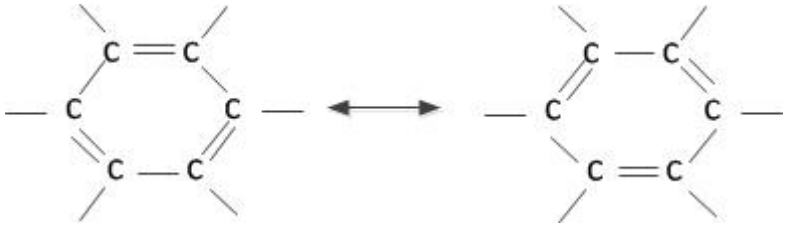


Figure 2-3 Benzene (one atom hydrogen joined with carbon atoms)

2.1.1.4. Unsaturated Aliphatic hydrocarbons or Alkene

Alkenes are also called Olefins or isoparaffins, are presented by branched or unbranched chains of carbons atoms. The molecular structures of Alkenes contain double-bonded carbon atoms. Unlike alkanes and naphthene which are abundant in crude oil, Alkens are not found commonly in crude oils (21), but they are mostly found in refining products. For instance, ethylene, propylene, and butene are the main raw materials for the petrochemical industry (24).

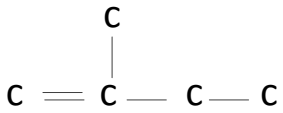


Figure 2-4. Structure of 2-methylbut-1-ene

2.1.2. Classification of Crude Oils

Based on molecular weight, crude oils can be classified into three groups-light, medium and heavy-weight components.

2.1.2.1. Lightweight components

Small molecules with one to 10 numbers of carbon atoms are characterized as light oils. Benzene and pentane are examples of this class (21). High volatility, readily dissolve and

evaporate are some of the properties of these components. They may be dangerous for the environment due to being flammable and readily inhaled (27).

2.1.2.2. Medium weight components

Carbon atoms range from 11 to 22 which have low volatility and solubility are in this class. These components are more complex than the low weight components. Due to low evaporation rate and their structure, they may remain several days in the environment (21).

2.1.2.3. Heavy weight components

Molecules comprise of more than 23 carbon atoms with the complex structure are defined as heavy oil. They are sticky or non-fluid oils which have lowest of evaporation. Heavy oils contain large amount of asphalthene (28) consisting of carbon, hydrogen, oxygen and sulphur, as well as trace amounts of vanadium and nickel (21, 29)

2.2. Refined products

The products that are obtained from oil and gas wells are composed of three phase of gas, liquid and solid. They should be separated for different usage. They have different boiling temperatures that can be useful for separation by fractional distillation. Physical and chemical properties of these products vary with distillation range and a number of carbon atoms. The most important product is petrol, which contains light and volatile components. Such components can quickly vaporize once being released into the environment, and thus does not present a persistent problems. On contrast, other products can create major problems by accidental releases.

2.2.1. Diesel

Diesel oil containing 11-21 carbon atoms (30) is obtained from crude oil distillation. Diesel oil is a mixture different normal, branched, cyclic alkanes, and aromatic compounds (31). The

boiling temperature of diesel oil is in the range of 180-300 ^{o}C (360-680 ^{o}F). Furthermore, low water solubility and low volatility are other properties of this product (10).

2.2.2. Paraffin oil

Paraffin oil that is a petroleum component that is composed of n-paraffin or alkanes (32) is called kerosene in the US and Canada, Australia, and New Zealand. It is a type of petroleum-based fuel commonly used in aircraft, where it is called jet fuel (33). The most important characterization of jet fuel is staying liquid even at the very low temperature (34).

2.2.3. Classification of petroleum refined products

American Petroleum Institute (API) gravity indicates the density of liquid petroleum products. The unit of API gravity is °API and can be calculated from specific gravity by the following equation.

$$\text{API Gravity} = (141.5 / \text{sp.gr}) - 131.5 \qquad \text{Equation 2-1}$$

Where sp.gr is the density at 60 ^{o}F (15.5 ^{o}C) compared with water density. API gravity of crude oil ranging from at 10 API to over 50 API can be used for the classification of crudes. The API gravity shows how heavy or light the oil is compared to water. The higher ^{o}API is shown the lighter oil. Refined products can be categorized into three classes as shown in Table 2-1.

Table 2-1. Refind oil classification (35)

API gravity	Oil classification	Example
°API < 22	Heavy	Paraffin oil
22 < °API <30	Medium	Naphtha
°API > 30	Light	Gasoline

2.3. Oil spillages and their effects

Industrializing and human activities are the main reasons of water contamination. The amount of oil spilled in the environment is a range between 1.7- 8.8 millions of ton petroleum hydrocarbon per year. Municipal and industrial wastes are the major reasons for this issue. For example, used motor oil leaked can be one the main contributor in water contamination. Another sources of oil spillages can be natural seepages, offshore production, and transportation which make a difficult estimation of oil input to the environment (36). Fortunately, the total amount of oil entering the environment not only has increased, but also has decreased over the last years due to the strictness of international requirement for the operations in offshore area (37). However, a number of incidents occurs every year. For example, in 2010, 60000 barrels of oil per day (38), and totally 795 million liters (39) entered in the Gulf of Mexico.

Oil spillages into the marine environments can result in catastrophic impacts as recently seen in the Gulf of Mexico (5). Oil spills may affect organisms in the marine area by disruption of biochemical or physiological activities of organisms and impairment in productivity. They also destroy animal immune systems and, as a result, kill them (40). According to (41), the adverse effect of oil spills can be deteriorated due to ultraviolet (UV) radiation. For instance, UV light causes an increment of PAH toxicity up to 50000 times (42).

The level of the impact of crude oil depends on amount and type of crude oil released. Toxicity of crude oil highly depends on PAH which are carcinogenic and mutagenic (43). Therefore, effects of oil spills depend on the chemical nature of the oil which may vary from source to source. Furthermore, the fate of oil spills is influenced by the volume of oil released, the climate conditions of spilled site including temperature and tidal intensity and oil-degrading microbes

(5). Therefore, when oil spills, it encounters different natural clean-up which can be discussed in the following section.

2.4. Natural Processes

Some environmental processes can affect oil spillages, which can be defined as weathering processes. These processes influence the fate of hydrocarbons in the environment. The following section represents weathering processes which can play an important role in the ultimate destination of spilled oil in the environment.

2.4.1. Spreading

Once oil is spilled, it distributes to the water surface quickly. The viscosity, volume of spilled oil and interfacial tension between water and oil can influence on spreading speed (21, 44). For instance, oils with low viscosity spread faster than oil with high viscosity. The other factors can affect on spreading are waves, tidal streams and currents (44), which can induce oil spreads across the water surface over several square kilometers just in few hours. When oil spillage accrues, oil spreads cohesively to form oil slicks and then begin to break up. The thickness of spilled oil can be varied from a micrometer to several millimeters (45).

2.4.2. Evaporation

Evaporation is a surface phenomenon, which depends on boiling point. In the case of oil spillage, oil compounds with low boiling points have high tendency to evaporate from the surface (21). Evaporation starts immediately after oil spills and may decrease more than half of oil volume during the one day. Evaporation does not decompose the oil. Instead it can transport them into air (26). Evaporation can reduce the toxicity and flammability of oil spillages, but enhances the viscosity and density of the remained oil (27).

2.4.3. Natural dispersing

Formation of oil droplets with the incorporation of the water column is the natural dispersion. The oil droplets are dispersed by advection and ocean currents (46). Natural oil dispersion depends on the oil droplet size, environmental condition, and oil components (21). For example, the light oil with small droplet size tends to disperse quickly in the water body. The large oil droplets (greater than 0.1 mm diameter) dispersed by breaking waves may join to other droplets and rise toward the surface, but the smaller one may mix into water body permanently (47). Dispersed oil makes faster natural degradation compared with a viscous oil slick.

2.4.4. Emulsification

A mixture of water droplets into spilled oil is emulsification process. Rate of emulsification depends on oil components and also sea state. Oil composed up Nickel/vanadium with 15 ppm concentration and asphaltene have a high tendency to emulsion form because of its molecular structure. Emulsification increase volume of oil residue in the environment and also prevent from another weathering process which can reduce the volume such as evaporation and dissolution (21, 27).

2.4.5. Dissolution

Spilled oil components may transfer from slick oil to the solution in the water column which is called dissolution. Dissolution is influenced by oil compositions, spreading, water temperature and dispersion. Lighter compounds such as benzene and toluene are slightly soluble, but they are virtually evaporated. As a result, only a slight fraction of oil (2%-5%) dissolves, and concentrations of dissolved hydrocarbons rarely exceed 1 ppm (47). In addition, dissolved oil may be transferred to other location by subsequent evaporation (48). Therefore, dissolved oil can be naturally removed without further applications.

2.4.6. Sedimentation

The sticky oil, particularly, heavyweight components of the oil tend to be buried at the bottom of the sea. Sedimentation can be accrued by one of the following items: (i) adhesion of oil to particles in the water column; (ii) deposition as fecal pellet after consumption by organisms; and (iii) sinking due to increase of density after weathering process. As a result of these changes, oil components cannot be influenced by additional weathering process (21). For instance, in Exxon Valdez oil spillage, oil buried in the shoreline particularly in cobbles remained unchanged for five years (49). Therefore, oil combination with sediments can be one of the main fates of spilled oil.

2.4.7. Biodegradation

One of the most important weathering processes is biological cleaning or biodegradation. Biodegradation is a natural process removing the remained biodegradable contaminants by microorganisms (11, 18, 26). Rate of biodegradation depends on bioavailability of oil contaminants. Bioavailability can be defined as the tendency of oil components to be decomposed by organisms (21). Generally, the biodegradability of lightweight components such as benzene is greater than medium and heavy weight hydrocarbons. Due to its relevance in this study, the bio-processes are discussed in length in the following sections.

2.4.7.1. Microorganisms

Microorganisms are the main factor in biodegradation and decomposition of the oil pollutants to less-toxic compounds such as CO_2 and water. There are various microorganisms which are able to eliminate oil contaminants such as bacteria, fungi, yeast and microalgae (14, 50, 51). Many researchers have focused on the exploration of the oil-degrading microbes which are the main factor in biodegradation, and many preview reports have been done to explore different

petroleum-degrading microorganisms (52-56). The first patent for a hydrocarbon degrading microorganism was generated in 1974 (57), and, in 1991, 70 species of microorganisms which can degrade oil contamination were reported.

Among different types of microorganisms, bacteria are the most predominant microorganisms in degradation of oil contaminants (10, 11, 58). In addition, numerous investigation has been conducted approaching the bioremediation capacity of bacteria due to the simplicity of culture, high amenability, and high capability to decomposition of different oil pollutants, (51). For instance, it has been noted that the main microorganisms for mineralization of diesel fuel are bacteria (10).

Oxygen plays the determinative role for the pathway of oil contaminants degradation. Consequently, bacteria have two mechanisms to decompose the oil compounds: aerobic and anaerobic.

2.4.7.2. Aerobic degradation

The complete degradation of the most of oil contaminants is conducted in the presence of oxygen or under aerobic conditions (59). Initially, Alkanes can be degraded by terminal oxidation or sub-terminal oxidation. The mono-terminal oxidation which is the main pathway yields an alcohol as an intermediated product. By further oxidation, aldehyde and then fatty acid are produced. Fatty acid undergoes β-oxidation to generate acetyl-CoA (60). The final products of oil degradation are CO_2, H_2O and cell biomass. During the aerobic degradation of n-alkanes, enzymes play a valuable role since the primary terminal hydroxylation of n-alkanes can be performed by them. As shown in Figure 2-5, NAD^+ is an enzyme which acts as a hydrogen acceptor.

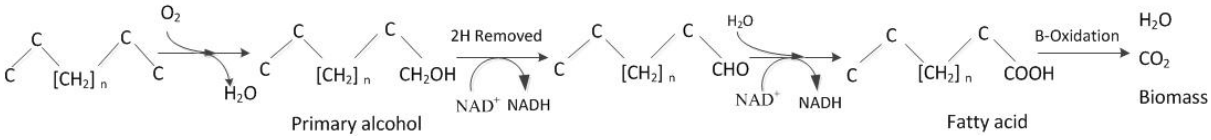


Figure 2-5. n-alkane degradation pathways, adapted from (61)

Generally, aerobic degradation of n-alkanes can be expressed by the following equations:

$$C_xH_yCH_3 \rightarrow C_xH_yCH_2OH \rightarrow C_xH_yCHO \rightarrow C_xH_yCOOH$$ Equation 2-2

$$C_xH_yCH_3 + \frac{3}{2}O_2 \xrightarrow{Bacteria\&Enzyme} C_xH_yCOOH + H_2O$$ Equation 2-3

2.4.7.3. Anaerobic degradation

Anaerobic degradation is essential for the decomposition of oil contaminants since oxygen is not available in many contaminated sites. The radical catalysed addition of fumarate to hydrocarbons results substituted succinate derivatives which are recognized reaction for degradation of alkyl-substituted benzenes (62). Degradation of Toluene is similar to this fumarate-dependant hydrocarbon activation. In this reaction, the main important factor is the enzyme benzyl succinate synthase. The generated benzyl-CoA which is a common intermediate product is decomposed via reductive dearomatization of the aromatic ring, hydrolytic ring cleavage and β-oxidation to acetyl- CoA units. The final oxidized products can include CO_2, CO and CH_4 (63).

Even though toluene and ethyl benzene are similar in terms of structure and chemistry, the anaerobic degradation of these two compounds is different. Ethylbenzene is hydroxylated and dehydrogenated to acetophenone, and, after carboxylation, converted to benzoyl-CoA (64).

There are numerous researches focusing on anaerobic degradation of benzene, toluene, ethylbenzene and xylenes (BTEX), but few studies have been conducted related to anaerobic

metabolism of n-alkanes because of slow anaerobic degradation of n-alkanes, poor solubility of the long chain n-alkanes and heterogeneous growth of cells in the medium (62). Furthermore, BTEX compounds are classified as most toxic and dangerous contaminants, which is major concern internationally.

Alkanes are the main constitute of petroleum and some are recalcitrant to decomposition. In the absence of oxygen, alkanes can be degraded in the complicated pathway. The first step in anaerobic degradation of alkanes is activation at the sub-terminal carbon of the alkane by the addition of fumarate, and as a result, alkyl succinate is formed. An anaerobic glycol radical enzyme, which is termed as alkyl-succinate synthase or (1-methyl-alkyl) succinate synthase, catalyse the alkyl succinate. After decarboxylation and β-oxidation, degradation results in CO_2 (62).

In addition to natural dispersion and degradation, the developments of different strategies are required to avoid contamination of clean area and also remove oil completely. Type of crude oil, the volume of spilled oil and environmental condition dictate the cleaning methods of the oil spillage as it is discussed below.

2.5. Physical cleaning methods

Physical methods whether manual or mechanical have been the method of choice in most instances to avoid contamination of a large area. Booms, skimmers, and sorbents are common tools for oil containment.

2.5.1. Booms

Booms control the oil from spreading over the water surface by floating on the surface. Due to their structures, oil cannot escape from the top or under the booms. However, this technique cannot be used in fast running rivers and large wind-swept lakes (65).

2.5.2. Skimmers

Skimmers are machines that physically suck the oil layer, along with some water. Consequently, the oil needs to be separated from water. A considerable amount of oil can be recovered with skimmers. However, this technique is not practical in many circumstances. For instance, skimmers are not recommended in spillages of heavy crude oil due to blockage of skimmers (66, 67).

2.5.3. Sorbents

Sorbents remove the oil based on the absorption or adsorption. Sorbents are hydrophobic materials and remove the oil from the surface of water easily. One of the drawbacks of this technique is sinkage of the oil-coated materials to the bottom in which it can endanger the organisms (27).

2.5.4. In-situ burning

Controlled in situ burning of an oil slick has been employed as one of the primary responses since the late 1960s (68). However, air pollution is the consequence of this technique.

2.6. Chemical cleaning methods

Even though physical methods have the capability of rapid removal of oil released, they are not able to remove oil completely (18), and also numerous supplementary aids are required. The most effective chemical cleaning method is dispersants. This method relies on the chemical interaction between dispersants and hydrocarbons. To assess the capability of dispersants in the removal of oil spillages and for identification, dispersants, and their main constitute are discussed in the following sections.

2.6.1. Surfactants

Surfactants or surface active agents are materials containing two heads of hydrophobic and hydrophilic groups (69). Due to their structures, shown in Figure **2-6**, they can solve in both organic solvents and water.

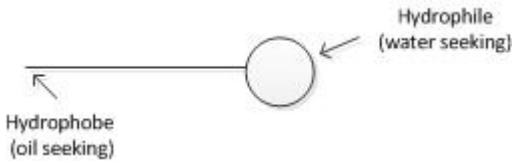


Figure 2-6 Surfactant molecule

Single molecules of surfactants are called monomers. Once the concentration of surfactants is increased, the free monomers accumulate to form the micelles. A concentration which monomers change to micelles is called critical micelle concentration (CMC). The increase of surfactants cannot influence on surface tension after CMC.

Surfactants are classified into four main groups of non-ionic, anionic, cationic and zwitterionic. Non-ionic surfactants are amphiphilic molecules which have no electrical charges. Anionic have negative charges hydrophilic group. These groups of surfactants are the most commonly used surfactants. In cationic surfactants, the hydrophilic group is positively charged. Zwitterionic or amphoteric have both negative and positive charges in their molecules (69, 70). Table 2-2 shows some examples of different kind of surfactants.

Table 2-2. Categorization of surfactants

Class	Examples
Non-ionic	Polyoxythylene alcohol
	Octylphenol Ethoxylate (Triton)
Anionic	Sodium dodecyl sulfate
	Sodium dodecyl benzene sulfonate
Cationic	Laurylamine hydrochloride
	Trimethyl dodecyl ammonium chloride
Zwitterionic	Dodecyl betaine
	Lauramidopropyl Betaine

Surfactants can solve in both organic solvents and water due to their structures shown in Figure 2-6. They adsorb to air-liquid surfaces and liquid-liquid interfaces to reduce the interfacial tension between water and oil (71).

2.6.2. Dispersants

Dispersants are composed of surface active agents such as surfactants, solvents and stabilizing agents (16). The precise composition of dispersants is commercially confidential, but the overall instructions specify two non-ionic surfactants and one anionic surfactant dissolved in a neutral nontoxic solvent (72). Surfactants in the dispersants accumulate at the boundary between the oil and water and reduce the interfacial tension. Consequently, oil slicks will break

and change to the oil droplets. Hence, dispersants can play a significant role in the distribution of the oil in the water column (21, 73) and dissipation of the oil slicks.

Dispersants can be classified into three groups (27):

- Type I dispersants mostly contains hydrocarbon solvent with one-fourth of surfactants. To apply this type, pre-dilution is required with the dose rate of 1:1 and 1:3 (dispersants: oil).
- Type II dispersants containing mostly surfactants has alcohol or glycol as a solvent. Predilection with sea water is needed with a dose rate of 1:10.
- Type III dispersants are formulated identical to type II products, and the dose rates are between 1:5 and 1:30 (neat dispersants: oil)

The effectiveness of dispersants in oil removal depends on oil composition, sea energy, climate condition, temperature, the salinity of the water, the type and amount of dispersants used (74). Among these factors, oil composition can influence mostly on dispersant application (75). For example, saturate hydrocarbons such as diesel fuel are dispersed by naturally spreading process and also dispersants. On contrast, resins, asphaltenes and most heavy hydrocarbons are hardly dispersible (42).

By increasing oil droplets in the water, which may contain PAHs, dispersion process can be toxic and unsafe for the environment (42). Hence, there are many arguments against the use of dispersants in the past five decades. For instance, the toxicity of dispersants was found in the late 1960s when dispersants damaged the sea life (74). After the catastrophic event, the composition of dispersants was changed to have less toxic effects. In the case of BP Deepwater Horizon spill, approximately 2.1 million gallons of dispersants were injected to the surface and well-head (5, 16), but the combined toxicity of oil and dispersant was reported two years after the spill (76).

Effectivity of dispersants in deep water depends on droplet size and also oil decomposition (16). Assessment of these two factors is challenging. However, there are two proposed methods for evaluating of the effectiveness of dispersants. First, dispersants dissolve in the gas phase in deep water, and consequently they become unavailable to the oil phase and dispersants are ineffective. Second, dispersants dissolve in the oil phase, which highly increase the surface area of the oil, and also accelerate oil removal.

In summary, physical and chemical methods can remove the oil rapidly (77) , but they are failed to remove the oil released in the environment completely. Furthermore, these methods are expensive and difficult to achieve sufficient oil removal (9). Therefore, the other method is required to solve oil spillages.

2.7. Biological cleaning methods

The technique of using biological agents to remove the contaminants from the environment is called bioremediation (78). This treatment stimulates microorganisms to accelerate to the removal of pollutants from the environment (18). In comparison with other methods, this technique has numerous advantages such as simplicity, cost-effectiveness. Most importantly, it poses minimal risks to contaminated sites (10, 51). Bioremediation techniques were developed and improved after oil spillages in Alaska in 1989 (26). The bio-technology attempts to facilitate microorganisms' growth by different strategies.

2.7.1. In-situ bioremediation

The method of in-situ bioremediation is used to make faster biological cleaning of contaminants by introducing of oxygen and nutrients to the contaminated sites without transportation of pollutants to the other location. This method is preferable when site conditions are not optimal for the natural growth of microorganisms (58). The capability of

microorganisms in decomposing the oil pollutants and also the development of more cells depend on nutrients and in some case oxygen (79).

2.7.2. Ex-situ bioremediation:

Ex-situ bioremediation, which aims to enhance microbial degrading, requires pumping of contaminated water or excavation of polluted site (58). In this method, contaminated soil or water transport into the other locations to treatment. If the contaminated site is small or surrounded by industrial or residential facilities, ex-situ bioremediation is preferred (27).

2.7.3. Bioaugmentation

The addition of microbial culture to expedite the degradation process can be defined as bio-augmentation. Bio-augmentation may be used to enhance the rate of biodegradation by the aid of native or exogenous bacteria. Native bacteria, which were adapted to the contaminated site, are more safe and efficient than exogenous bacteria which are introduced to the oil-polluted sites (10). However, lack of the enzymatic activity required for crude oil degradation sometimes limited the oil removal rates (9). Based on this reason, exogenous bacteria can be more suitable to different environmental conditions and induce a high rate of degradation (9).

Bio-augmentation with a mixed consortium of microorganism may be able to decompose different type of oil with complex structure. It is a well-established fact that single species of microorganisms cannot completely decompose all different components in the oil (9), which commonly exist in the polluted sites (58). Furthermore, one single bacterium cannot make all required enzymes to accelerate the biodegradation (9). The effect of salinity can be deteriorated due to applying bacteria individually to the contaminated area, but this inhibitory effect is reduced when a consortium of bacteria is used (80). Therefore, a consortium of

microorganisms, which produce a collection of enzymatic abilities, is required to have an effective bioremediation (9, 11, 52, 81).

2.7.4. Biostimulation

Microorganisms can grow in different environmental conditions, but the crucial factor for survival and mineralization of oil compounds are energy and carbon source (82). In marine areas, low amount of nutrients such as nitrogen and phosphorous were reported (9). These limitations can affect the rate of biodegradation (83). The addition of fertilizers containing nitrogen and phosphorous can be defined as biostimulation. It can significantly enhance the rate of biodegradation (5, 18, 58). However, nutrient amendment level is a crucial factor for bioremediation enhancement rate. Too high concentration can have a negative effect on biodegradation rate (84). On contrast, too low concentrations can be inadequate for the growth of bacteria (85). As a result, the optimum amount of fertilizer is required to be added to have an effective bioremediation.

2.7.5. Enzymatic bioremediation

Enzymes are comprised of hundreds of amino acids produced by living microorganisms. Microorganisms can generate Enzyme, which makes possible degradation of complex oil compounds. Enzyme responsibilities are to enhance the rate of a reaction along the desired pathway and also accelerate biodegradation process (86). Bacteria can decompose completely complex hydrocarbons such as PAHs in the presence of enzymes (5, 26).

One single enzyme molecule can transform many molecular transformations per second. However, to stimulate microorganisms, enzyme mixtures can be more effective than one single enzyme molecule in decomposing the complex oil contaminants (26). Furthermore, only a

small quantity of the enzymes are required for the catalysis of reactions since enzymes are not consumed in the reactions (86, 87).

Enzymatic reactions have numerous advantages such as costly effectiveness and being safe without producing the toxic secondary products. Moreover, the introduction of isolated enzymes does not generate toxic secondary products (88).

2.7.6. Biosurfactant

Biosurfactants are surface-active compounds generated by different microorganisms such as bacteria, fungi and yeast (89). The quantity, quality, and type of biosurfactants production depends on the type of microorganisms and the nature of the carbon sources (90). These biomolecules contain two ends of hydrophobic substrates and hydrophilic top. The hydrophobic head can be saturated, unsaturated, linear, branched or hydroxylated fatty acid and the hydrophilic top can be an amino acid, peptide, mono-, di- or polysaccharide (91). Hence, microorganisms can grow on the hydrophobic part and produce intermediate products such as fatty acids to decompose the hydrocarbon.

These compounds have many valuable applications in environmental protection. The most significant one is an increase of hydrocarbon bioremediation. Biosurfactants can enhance surface availability for microbial growth by the interaction between cells and hydrocarbon substrates (92). Biosurfactants reduce surface and interfacial tensions and as a result, increase the surface areas of insoluble compounds for the microbial attack (93). The most important characterization of biosurfactants making them unique are the capability to increase the bioavailability of poorly soluble compounds such as aliphatic hydrocarbon and polycyclic aromatic hydrocarbons (PAHs) (94). The solubility of aliphatic hydrocarbons decreases by increasing carbon numbers, and they are virtually water insoluble with a chain length of C_{12}-plus (95). However, they can be degraded by attachment of microbial cells at oil droplets and

the production of biosurfactants (61). Therefore, biosurfactants can increase solubility and consequently microbial availability of aliphatic hydrocarbon in the contaminated sites.

In contrast to synthesized surfactants which can be toxic, poorly degradable and accumulative in the environment (96), biosurfactants are non-toxic, biodegradable and better in environmental adaptability (58). In summary, biosurfactants are the key factor in the enhancement of microbial degradation of various hydrocarbons.

2.7.7. Limitation factors of biological cleaning

The effectiveness of bioremediation highly depends on microorganisms since they need to have the favorable condition to be able to degrade the complex hydrocarbon structure. Salinity, pH, temperature and oil microbial availability are the main factors can limit bioremediation.

2.7.7.1. Salinity

Microorganisms have the potential capacity for adaptation to different environmental conditions, but in extremely high salinities areas have been shown the low rate of biodegradation (21). The inverse relationship between the salinity and growth of bacteria has been reported (97, 98). Microorganisms in the marine area can survive for a certain range of salinity. For example, low biodegradation rate of PAH compounds has been observed in the medium with high salinity (80, 99).

2.7.7.2. PH

In biodegradation process, neutral pH (6.5-7.5) has been reported to be suitable for growth of most bacteria such as heterotrophic bacteria (100). Acidic environments limit the enzymatic activity and effectiveness of biodegradation. For example, slow rate of biodegradation has been observed in salt marshes with pH of approximately 5 (101).

2.7.7.3. Temperature

Biodegradation rate can be various in sea water because of temperature range which is between -2 and 35ºC (101). Temperature affects on oil composition and microorganisms and as a result on biodegradation rate (75). The rate of biodegradation decreases at low temperature due to a decrease in oil volatility of light hydrocarbon, an increase of oil viscosity, less spreading and less surface microbial availability (101). Furthermore at high temperature, microbial degradation decrease due to heat stresses (21). As it is shown by (102), mesophilic temperature (typically 20-45ºc) can be more favorable for an increase in biodegradation rate.

2.7.7.4. Microbial availability of oil

Even though bioremediation is the most effective method of oil removal, Poor microbial availability is the important factor which may limit the effectiveness of bioremediation. Biodegradation rate of dissolved oil is higher than dispersed oil droplets since there is no interface limitation (17). Since most of the hydrocarbons are insoluble in water (5), adjustment of elements leading an increase of oil-water interface can significantly increase oil biodegradation.

The following new method can increase the oil-water interface and address the most important reason for the ineffectiveness of bioremediation.

2.8. Floatation of water droplets on oil surface

Surface tension is the main factor in floating of different objects on the liquid surface. It can work against gravity and support solid objects and liquid lenses (103, 104). Recently, floatability of heavier liquid droplets (water) on lighter liquid (oil) has been verified numerically and experimentally (19). This technique can have a significant influence on

biodegradation and increase of microbial oil availability (105). Different interfacial phenomena can influence on floatation of heavier droplets on a lighter drop which is discussed in the following parts.

2.8.1. Capillary phenomena

Capillary phenomena emerge due to the interfacial tension between liquid and fluid. The curvature surface at the boundary of liquid with another fluid leads to the development of pressure which can be expressed by the Laplace equation.

$\Delta P=P_1-P_2=\gamma J$ Equation 2-4

Where P_1 and P_2 are pressures in two phases, γ is the interfacial tension (105).

In the above equation, J is the curvature of the interface which has principal radii, R_1 and R_2 (106):

$$J=\frac{1}{R_1}+\frac{1}{R_1}$$ Equation 2-5

Therefore, this phenomenon is linked with the interfacial tensions which can influence on floatation of the droplet.

2.8.2. Interfacial tension

Surface or interfacial tension can be defined as force per unit length or energy per unit area. It can decrease the interfacial area from energy and, as a result, a droplet can float on the other fluid. The molecules are in the interface have unbalanced Vander Waals forces leading the transformation of molecules to the bulk liquid (105). Hence, the droplets tend to form a spherical shape to reduce interfacial tension.

2.8.3. Contact angle

The contact angle between the interfaces may influence the final shape and stability of the floating water droplet on oil surface. Determination of contact angles between fluid/fluid interfaces on a solid surface has been studied extensively (107, 108), but fluid/ fluid/fluid systems (Figure 2-7) which is the fundamental of this study are limited (19). Water droplet can float on oil surface in air medium when following from the Neumann's triangle.

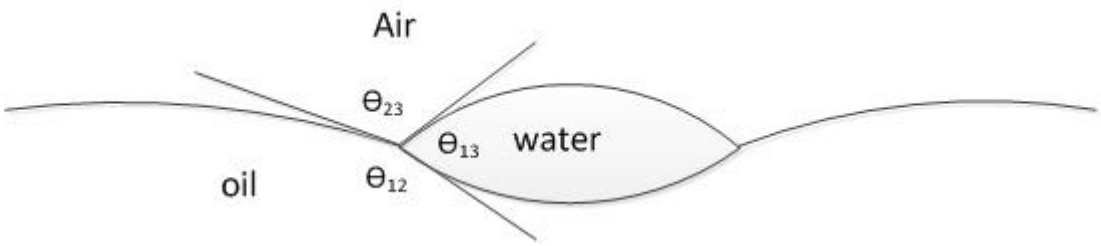


Figure 2-7 Three phases of air, water, and oil with different contact angle between interfaces (109)

Neumann's triangle that satisfy the equilibrium configuration in a three phases system (109) can be showed in Figure 2-8.

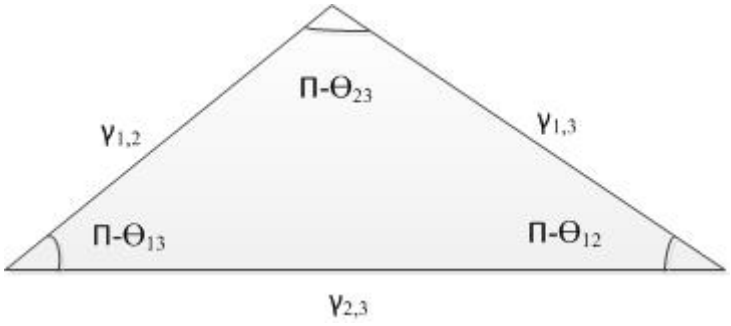


Figure 2-8. Neumann's Triangle

Neumann's triangle presents the relation of three interfacial tensions. It shows the sum of the two tensions needs to be more than the third tension, otherwise, there is no longer a line of three-phase contact (109, 110). Therefore, the boundary conditions of these equations are constrained by the contact angles.

2.8.4. Numerical model of floatation of heavier droplets in a lighter liquid

The physical properties involved in the floating water droplet on oil surface are densities of air, water and oil, and also three interfacial tensions between three phases (γ_{wa}, γ_{ow}, γ_{oa}). These parameters are fixed in the model to be able to find the vertical forces on floating water droplets (19). Vertical forces on water droplets floating on oil surface (Figure 2-9) are determined using Equation 2-6.

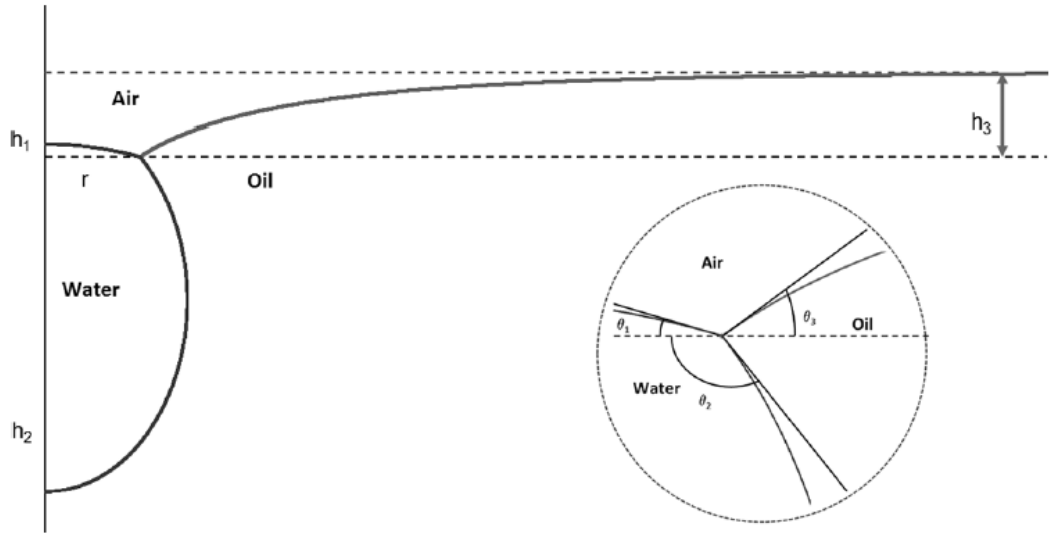


Figure 2-9. Diagram of a water droplet on oil surface.

The three interfaces are governed by the Young−Laplace equation and matched at the contact line, radius r. The three contact angles are arranged according to Neumann's triangle so that the sum of three tensile forces acting on the contact line is zero (19):

$$F = g[V_b\rho_w + (\pi r^2 h_3 - V_1)\rho_a - (\pi r^2 h_3 - V_2)\rho_O]$$ Equation 2-6

Where r is the contact line radius, ρ is density, h_3 is the height of the droplet, V_1 and V_2 are the volumes of air/water and water/oil sections, respectively ($V_1 + V_2 = V_b$). In this equation, the first two terms are gravitational forces and the last term is an upward force. H_3 is found from Equation 2-7 and Equation 2-8 where γ is interfacial tension.

$$\theta_1 + \theta_2 = \pi - \arccos[\frac{\gamma_{wa}^2 + \gamma_{ow}^2 + \gamma_{oa}^2}{2\gamma_{oa}\ \gamma_{wa}}]$$ Equation 2-7

$$\theta_1 + \theta 3 = \arccos[\frac{\gamma_{wa}^2 + \gamma_{ow}^2 + \gamma_{oa}^2}{2\gamma_{oa}\ \gamma_{wa}}]$$ Equation 2-8

These two equations are derived from Neumann's triangle. Neumann's triangle relating the three interfacial tensions at the contact angles of three fluid phases is fundamental to this model.

The floating droplet model was verified experimentally (19). The theoretical model indicated that droplet stability depends on the combination of three interface tensions, oil density, and droplet volume.

2.8.5. Changes in interfacial tension and stability of floating droplet

Different factors can decrease surface or interfacial tension and consequently increase the stability of water droplets on oil surface.

2.8.5.1. Influence of surfactants on interfacial tension

Surfactants which have two heads of hydrophobic and hydrophilic groups are accumulated at the interface and increase force acting against the normal interfacial tension and consequently expand the interface (71). In addition to synthetic surfactants, naturally occurring surfactants such as asphaltenes can be very strong surfactants (111). They can decrease interfacial tension effectively (69). Effect of surfactants on the stability of water droplet on oil surface has been

verified and also has been reported that a water droplet containing surfactants are more stable to disturbance compared with the water droplet (20).

2.8.5.2. Influence of electrolytes in interfacial tension

Sodium Chloride (NaCl) plays an important role in the reduction of surface tension. NaCl in the presence of surfactant can decrease CMC and surface tension of surfactant solution. Furthermore, it decreases the solubility of surfactant and consequently reduces the ionization. Therefore, surfactants adsorb effectively at the oil-water interface resulting interfacial tension reduction. Moreover, in the presence of salt, the tendency of surface active agents is accumulation in the interfaces. This effect can be identified as salting out phenomena which aid to decrease surface tension (112).

2.8.5.3. Influence of pH in interfacial tension

The pH is one of the factors in changing the surface tension since it can influence the amount of surface active components (112). This effect in interfacial tension varies and depends on the chemical reaction and crude oil components**.** The following equations show the dissociation of acidic and basic components leading production of surface-active components.

$HA \leftrightarrow H^+ + A^-$ Equation 2-9

$BOH \leftrightarrow B^+ + OH^-$ Equation 2-10

Where HA and BOH represent acidic and basic components in crude oil, A^- is the surface-active ion and H^+ is the hydroxyl ion.

At high pH, surface-active ions are produced and consequently reduce interfacial tension. Furthermore, at low pH, the basic component induce to decrease the interfacial tension (103).

2.8.5.4. Influence of bioprocess in interfacial tension

Effect of bioprocess in floatability of a water droplet on oil has not been explored. However, in the biological process, during the interaction with the organic material, biosurfactants which are one of the microbial approaches for degradation are produced. Biosurfactant can decrease surface tension effectively due to their structure, and they may increase the stability of a floating water droplet on oil surface. The other microbial method in bioprocess which may influence on interfacial tension is biofilm. Biofilm is assemblage and attachment of microorganisms to surfaces or interfaces (113). During biodegradation process, bacteria encounter to different environmental conditions such as UV fluctuations, nutrient starvation, metal toxicity and environmental temperature changes overcoming by biofilm formation (114-116). Formation of biofilm and attachment to surfaces depends mainly on surface structure and hydrophobicity since microbes tend to form biofilm in rougher and more hydrophobic surfaces (117). Therefore, biofilm and biosurfactants in interfaces may influence on interfacial tension and floatation of water droplets on oil.

2.8.6. Evaporation

Bacteria need water to decompose the oil compounds to less toxic components as shown in Figure 2-5, but water evaporation influence on this process. On the other hand, bacteria may produce biofilm or surfactants on air/water interface, and such films may/may not affect the evaporation rate. Many researchers have studied the rate of evaporation from liquid droplets, sessile or suspended in air (118, 119).

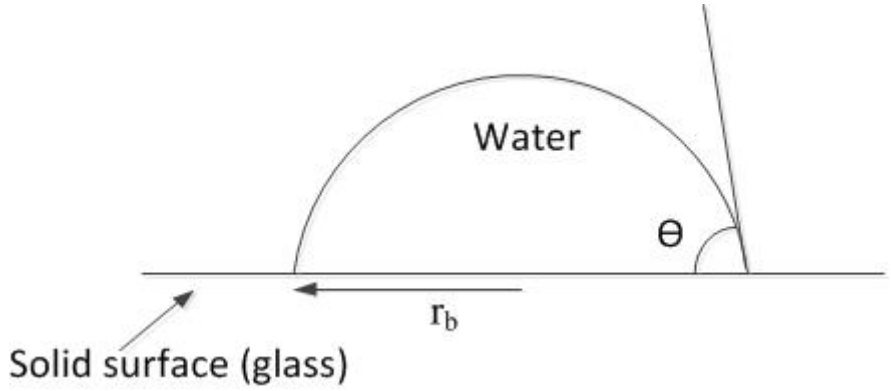


Figure 2-10.Water droplet on a mildly hydrophobic condition (Θ ~ 41°)

The rate of evaporation of a sessile droplet depends on the contact angle between the droplet and the solid substrate (Figure 2-10) and can be expressed by the following equation (120):

$$rate = I = \frac{-dm}{dt} = \rho(\frac{dv}{dt}) \qquad \text{Equation 2-11}$$

Where *m* is the mass of droplet, *v* is the volume, ρ is the density of liquid and *t* is the time.

In addition, if the rate of evaporation is dependent on contact radius, then the rate of evaporation can be expressed by Equation 2-12.

$$rate = -\rho(\frac{dv}{dt}) = a_1 + a_2(r_b) \qquad \text{Equation 2-12}$$

Therefore, the rate of water evaporation can be 9.21×10^{-5} (gr/min.mm) × r_b (mm) (120). It was reported that the evaporation rate of a water droplet on a solid surface is constant, and it depends on the hydrophobicity/ hydrophilicity of the solid surface.

Different factors can influence evaporation rate. It has been found that the evaporation rate of droplet containing pentadecanoic acid (PDA) is not altered by the presence of surfactant. Yet, surfactants can be favorable in the direction of particles generating from evaporation (121). However, it has been noted that monolayers composed of molecules with two heads of hydrophilic and hydrophobic (122) can reduce evaporation rate (123). Fatty acids, esters, and alcohols can form monolayers at the water/air interface and delay the evaporation of water (124). To reduce effectively the water evaporation and, a mixture of polymers and long chain

alcohols monolayer materials has been employed (124, 125). Effect of salt in changing the evaporation rate has been also verified. The evaporation rate is expectedly reduced by addition of salt due to the decreased vapor pressure at the water surface (126). It was reported that biodegradation process can influence the rate of evaporation (124).

In summary, the evaporation rate affect floating droplet since other factors (salt, acids, alcohols, and bioactivity) can reduce evaporation, and it is expected these factors affect a floating water droplet.

2.9. Summary

Oil spillage and it impacts on the environment has been raised international concerns. Many types of research have been conducted to find an effective method for oil spill removal. Oil spillage can be contained and removed by different methods, but environmental conditions and type of crude oil released affect these methods. Physical and chemical methods are expensive and can produce undesirable consequences. Bioremediation can be the best technique in the removal of oil spills since it does not produce secondary risks. However, poor microbial availability limits the effectiveness of this treatment.

The floating water droplet is the newly discovered phenomenon which can address this limitation. This method, which can be combined with chemical and biological methods, lays the foundation for this study.

Droplet floatation depends on oil density, droplet volume and interface tensions between the oil, water, and air. During a biodegradation process, biofilm, biosurfactant, alcohol, aldehyde and fatty acids are produced which may affect on surface tension and volume of the floating droplet, and consequently, influence on stability and shape of a floating droplet. However, experimental verification of such combinations is not available.

This study will present the new strategy in oil removal and address the bioremediation limitation and present the following questions.

- Influence of bacteria on stability of water on oil surfaces
- Influence of intermediate product during biodegradation in floatability of water droplets
- Effectivity of this new method in biodegradation process and oil decomposition

By successfully addressing these three questions, the project will verify optimization of bacterial growth on crude oils. Finally, this method can be used in the degradation of Diesel oil which is difficult to be decomposed due to its complex structure.

Chapter 3: Methodology

This section describes the materials and procedures. In addition, instrumentation which has been used in this study to measure properties of materials will be discussed briefly.

3.1. Materials

3.1.1. Chemicals

The main surfactant for this study is sodium dodecyl sulphate (SDS), which was obtained from Sigma-Aldrich at 99% purity. Triton X-705 solution(x70570), where obtained from Sigma-Aldrich, was also used to verify the effect of nonionic surfactant in the process.

Sodium chloride (NaCl) used to simulate seawater was purchased from Sigma-Aldrich. Paraffin oil from Digger Inc., Australia was used to be representative of crude oil. Crude oil spillages subject to evaporation and dissolution and, as a result, the larger hydrocarbons remain. Thus, paraffin oil which contains 16 to 20 carbons can be similar to crude oil spillages. Moreover, its transparency facilitates the optical verification of droplet behaviour during the experiment. Hence, the bio-degradation of paraffin oil can be a good model for verification of our new method.

In addition, other materials were used to verify the process at different variations. These include a variation in term of nutrient source and stabilization surfactant. Furthermore, diesel was also tested. Diesel oil is one of derivatives of crude oil which resistance to biodegradation due to its complex structure and low solubility in water (10). Diesel oil was purchased from BP Australia RTY Ltd.

3.1.2. Enzyme and nutrient

An Enzyme solution was obtained from Enzyme Wizard PTY LTD, Australia. The enzyme solution is currently employed, as the nutrient source, to enhance bio-degradation of oily waste on industrial sites around Australia.

In addition to enzyme solution, Modified Leonian's agar (MLA) medium was prepared which will be explained in the following section.

3.1.2.1. MLA medium preparation

In this study, MLA was prepared to verify the effect of this medium to bacteria and consequently, stability and floatability of a droplet on oil surface.

Filter-sterilisation prepared the MLA medium. All nutrients, vitamin, and micronutrients were filtered by using a 0.22 mm filter and added into 250 ml Schott bottle. Sodium bicarbonate ($NaHCO_3$) and Calcium chloride dehydrate ($CaCl_2$) were sterilized into an Autoclave. Autoclave – sterilized $NaHCO_3$, $CaCl_2$ and filter-sterilized nutrients solution were then added aseptically in a sterile 1000 ml Scott bottle and mixed well.

All the chemicals were used for the preparation of medium is tabulated in Table 3-1.

Table 3-1. Composition of MLA medium

Name	Formula	Final Concentration (mg/l)
Sodium bicarbonate	$NaHCO_3$	16.9
Calcium chloride dihydrate	$CaCl_2.2H_2O$	29.4
Nutrients		
Magnesium sulphate heptahydrate	$MgSO_4.7H_2O$	49.4
Sodium nitrate	$NaNO_3$	85

Potassium phosphate dibasic	K_2HPO_4	6.96
Boric acid	H_3BO_3	2.47
Selenious acid	H_2SeO_3	0.0012
Vitamins		
biotin	$C_{10}H_{16}N_2O_3S$	0.0005
Vitamin B12		0.0005
Thiamine HCl	Thiamine HCL	0.1
Micronutrients		
Disodium EDTA	Na_2EDTA	4.36
Ferric Chloride Hexahydrate	$FeCL_3.6H_2O$	1.58
Sodium bicarbonate	$NaHCO_3$	1.2
Manganese Chloride tetrahydraet	$MnCl_2.4H_2O$	0.36
Copper sulphate pentahydrate	$CuSO_4.5H_20$	0.01
Zinc sulphate heptahydrate	$ZnSO_4.7H_2O$	0.022
Cobalt chloride hexahydrate	$CoCL_2.6H_2O$	0.01
Sodium molybdate dihydrate	$Na_2MoO_4.2H_2O$	0.006

3.1.3. Microorganism

In this study, hydrocarbon degrading bacteria, which were a mixture of 60 species, were purchased from Solutions Unlimited Australia Pty Ltd. Trade name of this product is Grease Gone which has been approved for commercial and domestic usage in Australia, Malaysia and New Zealand. Due to high ability of a consortium of bacteria compared with single bacteria, the mixture was used for this study (18).

3.2. Preparation of aqueous solutions

Deionized water was used for the preparation of solutions. A sachet of 7 g of dried bacterial materials was dissolved in 800 ml of deionized water (Milipore, 18 M). The heater was adjusted at 40 ^{o}C, container containing bacteria and water was thoroughly mixed with a magnetic stirrer. Subsequently, 2 ml of bacteria solution was added to a 50 ml enzyme solution and left for 24 hours to have enough time for saturation. A solution of 0.01 M SDS, which is above CMC, was made. SDS solution was made on the same day that each experiment was to be conducted to prevent from natural hydrolysis. A 3.5 % NaCl was dissolved in deionized water to prepare synthetic seawater. Finally, 3.5 wt % NaCl, 0.01 M SDS and enzyme/bacteria were mixed to have the intended solution.

To ensure the reliability, all measurements were repeated three times.

3.3. Measurements methods

The main parameters in this study were measured by different devices which will be explained. Also, the procedure for detecting water droplet to measure surface tension, volume, contact angel and also radius will be described. Experimental part of this study consists the following sections:

- Measurements of properties: density, pH, surface tension
- Droplet shapes analysis
- Further verification (Evaluation of different surfactant, medium and environment on floatation of water droplet containing bacteria)

All physical measurements were conducted twice and at room temperature of 25^{o}C for result reliability.

3.3.1. Physical properties

3.3.1.1. Liquid density

The density of Paraffin oil, Diesel oil, water, and solution were measured by using a DMA 4500 Instrument (Anton Paar), as can be observed in Figure 3-1.

Figure 3-1. Anton Paar Densitometer

The density of materials was measured three times, and an average of data was tabulated in Table 3-2.

Table 3-2. Physical properties of the material (measured at 25ºC)

Material	Density (g/cm3)
Pure water	0.9954±0.02
Solution	1.0442±0.02
Paraffin oil	0.8307±0.02
Diesel oil	0.83173±0.02
Air	0.001225±0.02

3.3.1.2. Solution pH

Acidity and alkalinity affect bacteria growth, so the solution pH must be measured and adjusted accordingly. Since enzyme can work in the pH range of 3 to 8.5 and pH of the solutions was about 7.13-7.24 (Table 3-3), pH adjustment was not necessary.

Table 3-3. pH of different solutions

Solution	pH
NaCl, SDS	7.13±0.04
Enzyme, SDS, NaCl, bacteria, pure water	7.2±0.04
Enzyme, Triton, NaCl, bacteria, pure water	7.24±0.04
MLA medium, SDS, NaCl, bcateria, pure water	7.2±0.04

3.3.1.3. Surface tension

Surface tension is a very significant factor in floatability of a water droplet on oil surface. The following sections represent a different method for measurement of surface tension.

Maximum bubble pressure method

Maximum bubble pressure method is a technique for measuring the surface tension of a solution containing surfactants. MPT Lauda tensiometer was used to measure surface tension between air and water as shown in Figure 3-2.

Figure 3-2. MPT Lauda tensiometer

An equilibrium surface tension is 25 mN/m as shown in Figure 3-3.

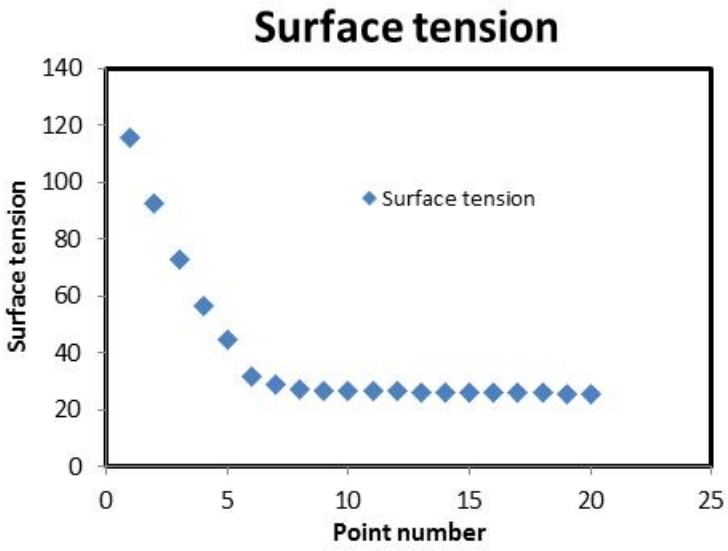


Figure 3-3. Dynamic surface tension (water solution/oil)

Pendant drop method

The pendant and sessile drop shape analysis methods are effectively used to measure surface tension. Axisymmetric Drop Shape Analysis (ADSA) is the most powerful technique for this measurement (127-129). Images are analyzed by fitting the drop profile with the Young-Laplace expression to estimate the value of the surface tension.

Following steps are required in determination of interfacial tension by using ADSA technique:

1. Image acquisition: images were recorded from the side of the cuvette by using autofocus camera.
2. Image preparation: To use ADSA, images were cropped by using Image J™ (www.imagej.net) and also all images were converted to tiff images.
3. ADSA inputs: physical properties and a scale factor of the images in the involved system were considered.

Image acquisition

Pendant drop method was used to measure surface tension between air and water to check the data gathered by using maximum bubble pressure method. All images were modified to be compatible in ADSA which can be observed in Figure 3-4.

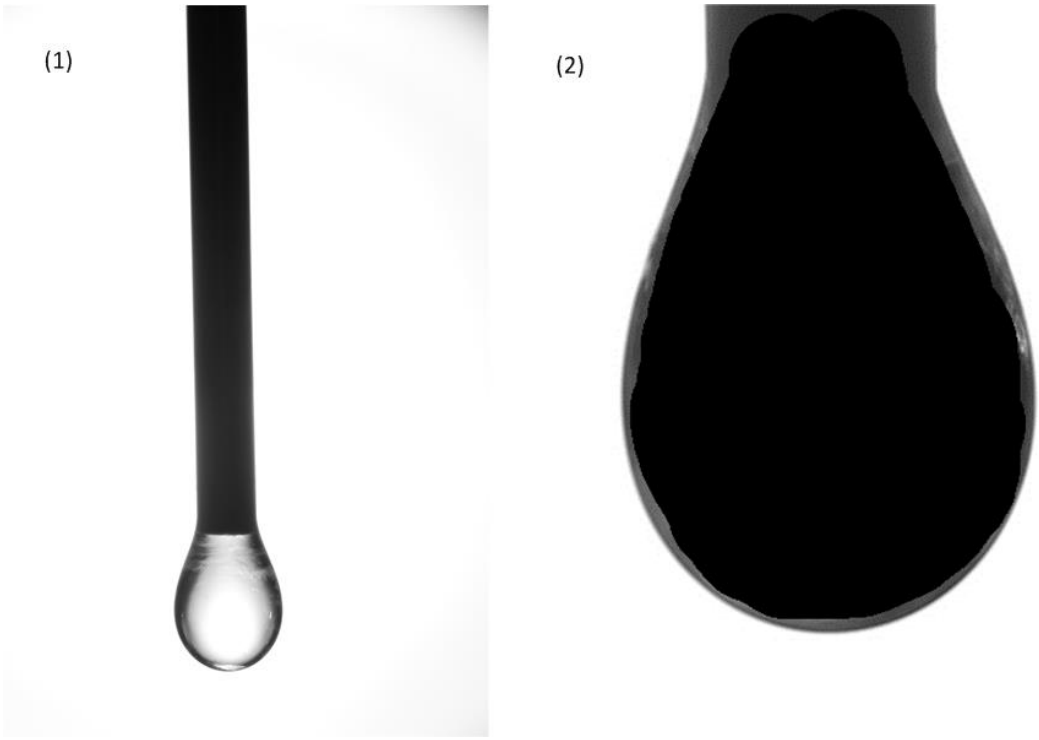
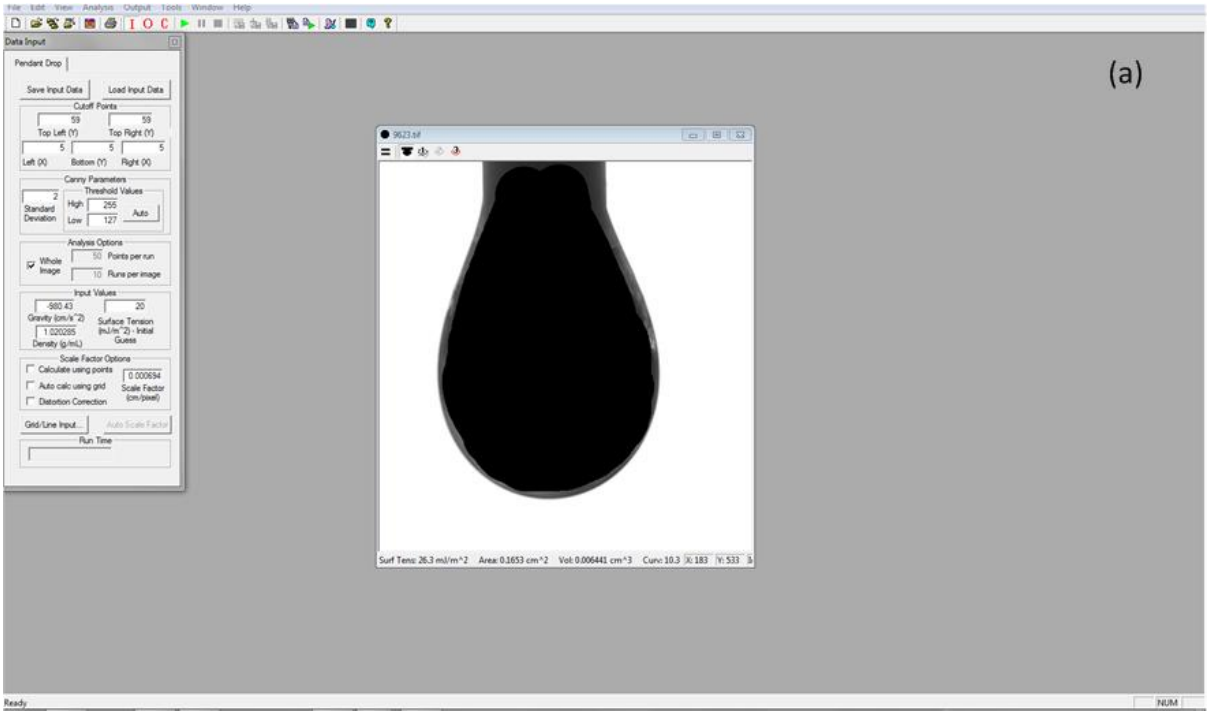


Figure 3-4. Image acquisition (1) and Image preparation for using ADSA (2)

To obtain the surface tension between the air/water interfaces, the ADSA method was used. Figure 3-5(a) shows drop profile used to measure interfacial surface tension and Figure 3-5(b) displays the results.



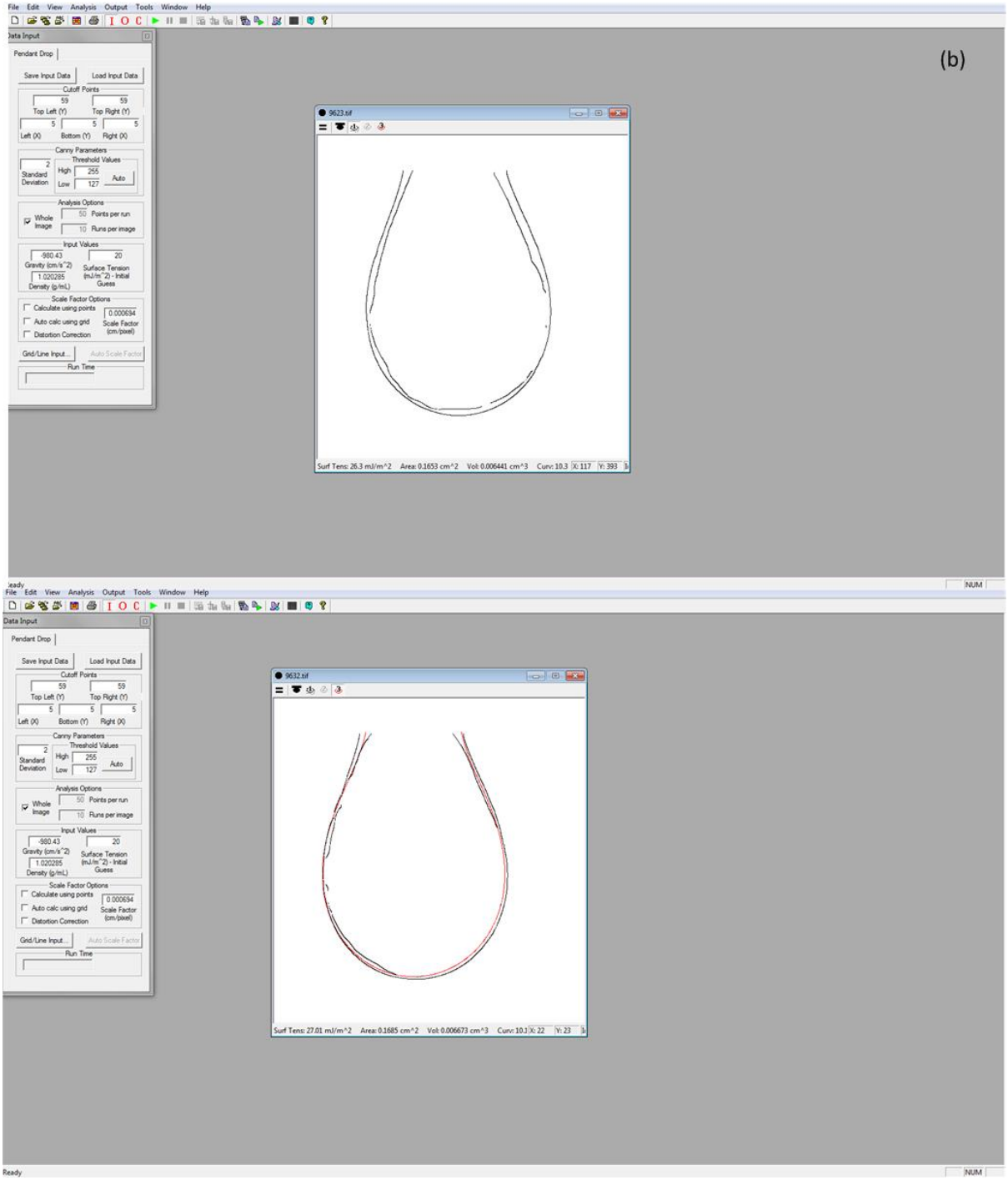


Figure 3-5. (a) ADSA software for measuring the surface tension (b) ADSA software after proceeding

3.3.2. Droplet shape analysis

3.3.2.1. Experimental setup

A cuvette was filled with aqueous solution and a thick layer of paraffin oil (~ 1 cm). One droplet of prepared solution was deposited on the oil/air surface. The shape of the droplet was monitored with a digital camera, with 3X magnification as shown in Figure 3-6 (a).

Furthermore, the video of a droplet containing bacteria was recorded from the top by using a digital microscope (A005+) with 100x magnification and includes a built-in 5-megapixel camera. The microscope was connected with USB cable to the computer as shown in Figure 3-6.

To verify the effect of evaporation on the droplet and also fully comprehend the effect of bacteria on the droplet, this experiment was repeated without bacteria and enzyme. Subsequently, the images were analyzed to obtain the contact radius and contact angle (i.e., r and θ_2).

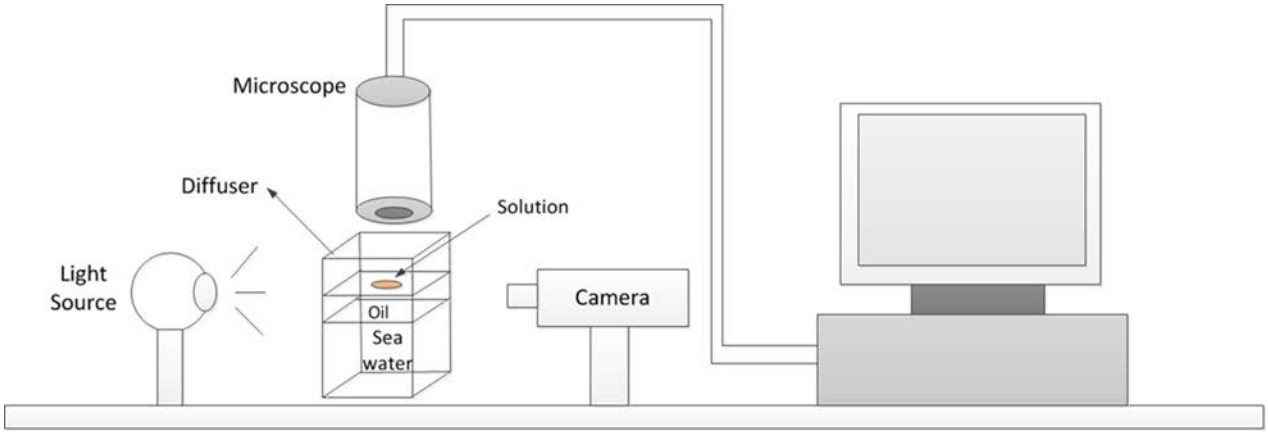


Figure 3-6. A picture of actual experimental setup

3.3.2.2. Image processing

The edge of the droplet was detected by using MATLAB software. For the MATLAB function to be able to produce the accurate edge of the droplet, all images were cropped as shown in Figure 3-7 (b).

Figure 3-7. (a) Deposition process. (b) Image cropped

The cropped images were uploaded into MATLAB, and the code for edge detection was entered as shown in Figure 3-8.

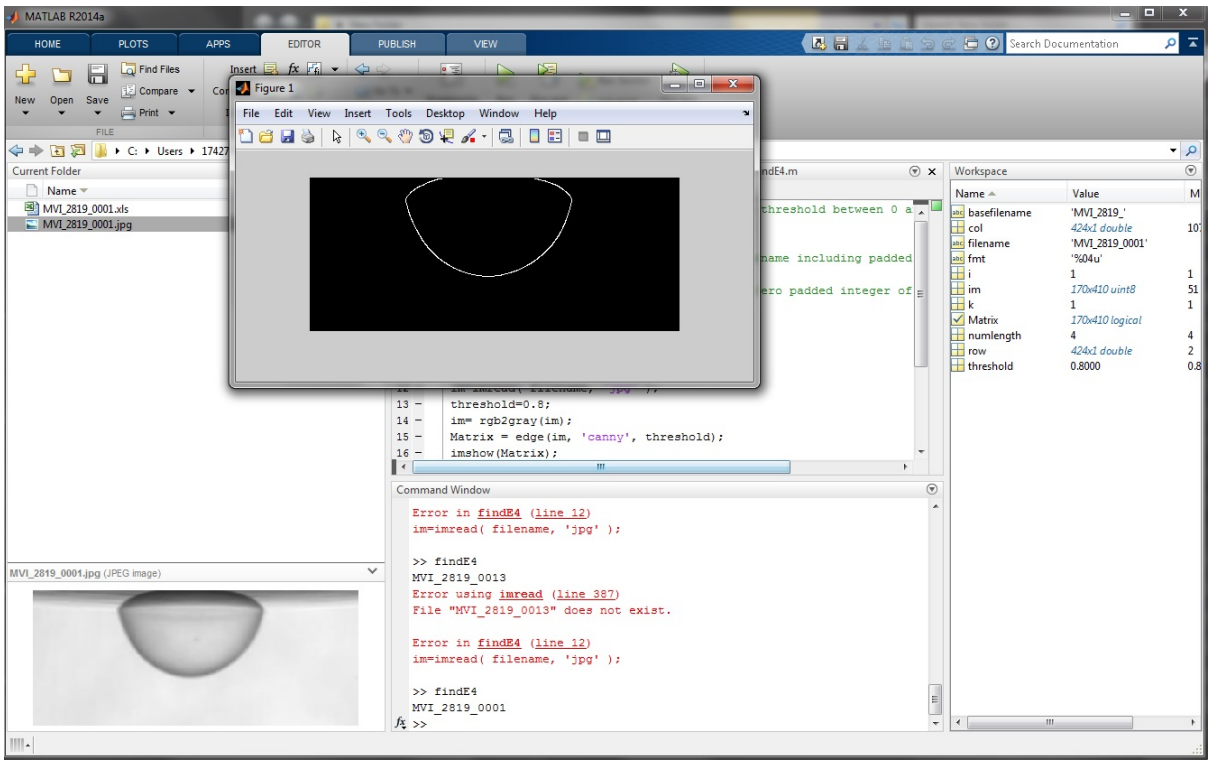


Figure 3-8. Program code.

The edge of the droplet was obtained which can be observed in Figure 3-9. Details of the code can be found in Appendix B.

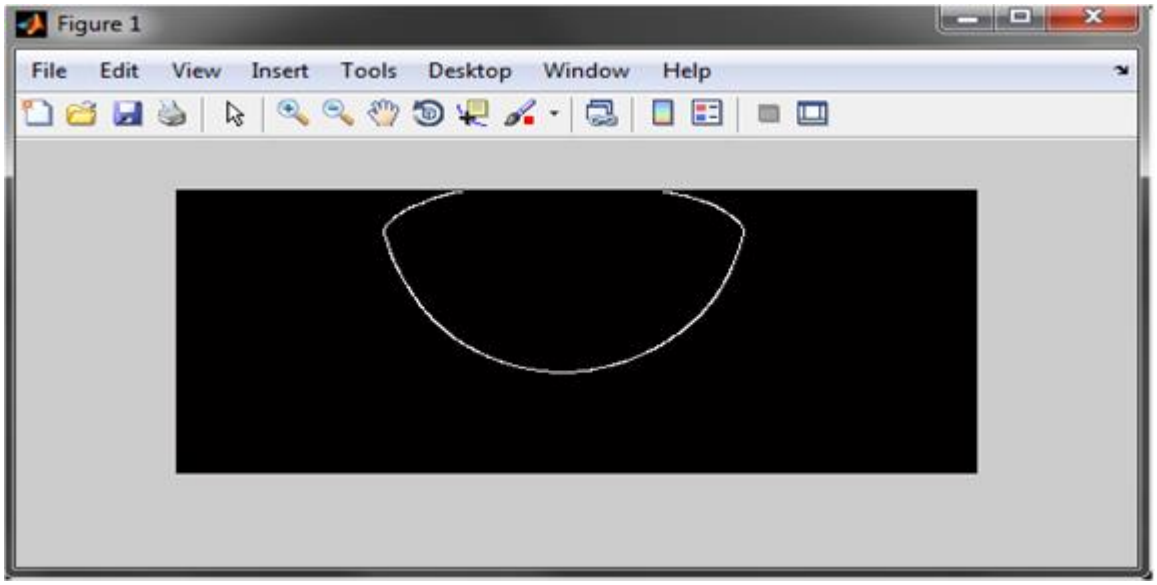


Figure 3-9. Edge detection to estimate a fit droplet shape

Subsequently, the profile of the droplet was exported to an Excel file for the best edge fitting. The shape of the droplet is described by the following equation:

$$y_{poly} = a_0 + a_2(x-b)^2 + a_4(x-b)^4 + a_6(x-b)^6 + a_8(x-b)^8 \qquad \text{Equation 3-1}$$

a_i and b are mathematical constants parameters, y and x are the vertical and horizontal coordinates of the droplet shape respectively.

The values of a_i and b for each droplet were obtained by fitting the edge to Equation 3-1. By applying this polynomial function, the profile of the droplet can be described mathematically as shown in Figure 3-10.

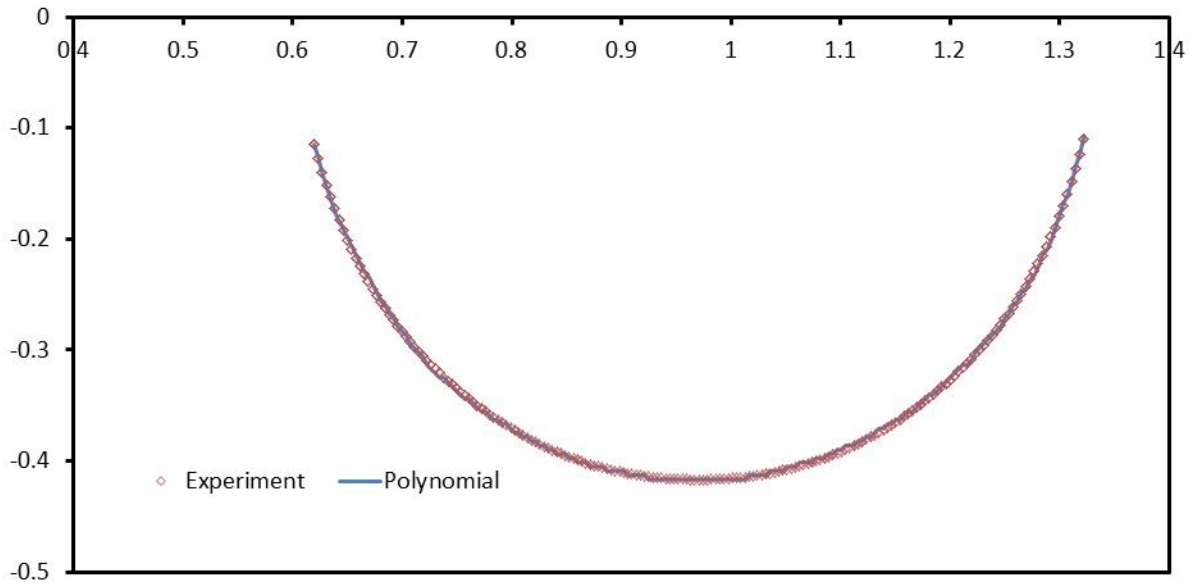


Figure 3-10. Edge profile of water droplet by applying polynomial function

Consequently, contact angles and droplet volume were calculated. The contact angle between the droplet and oil layer (rad) can be determined by:

$$\tan\theta_{2(right)} = \frac{dy}{dx}_{x=x_{\max}} = 2a_2(x-b) + 4a_4(x-b)^3 + 6a_6(x-b)^5 + 8a_8(x-b)^7 \qquad \text{Equation 3-2}$$

$$\tan\theta_{2(left)} = \frac{dy}{dx}_{x=x_{\min}} = 2a_2(x-b) + 4a_4(x-b)^3 + 4a_6(x-b)^5 + 8a_8(x-b)^7 \qquad \text{Equation 3-3}$$

Where θ_2 (right) and θ_2 (left) represent the right and left contact angles respectively, X_{max} and X_{min} are the horizontal limits of the contact line as shown in Figure 3-11.

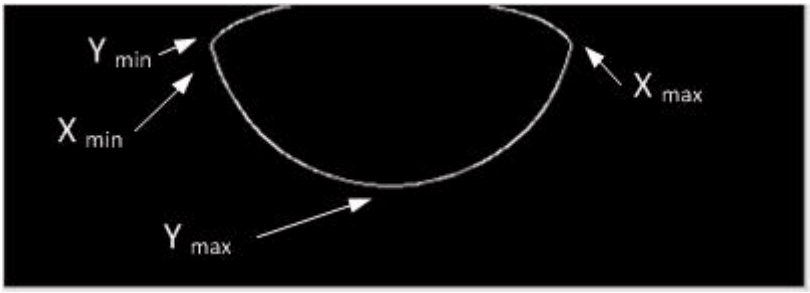


Figure 3-11. Edge of droplet detected by MATLAB

The numerical integration was used to calculate the droplet volume as:

$$V = \int_{y\min}^{y\max} \pi x^2 dy$$ Equation 3-4

Scale factor

To use the polynomial function for measuring the radius, contact angle and volume, scale conversion (pix/mm) was required. For determining the scale conversion, two points with defined distance was considered. By using Image J™ (www.imagej.net), coordinates of the two points was recorded and replaced in equation 3.5.

Conversion factor = $\sqrt{(y_2 - y_1)^2 + (x_2 - x_1)^2}$ Equation 3-5

3.4. Material variation

In addition to the standard solution, following changes were applied to understand the influence of materials on the method:

- Enzyme was replaced with the prepared MLA medium.
- Nanoionics surfactant (Triton) also was substituted of anionic surfactant (SDS) to investigate the effect of these surfactants on bacteria and floatation of water droplet.
- Diesel oil was replaced with paraffin oil to verify the effect of different oil on floatation of water droplet.

Chapter 4: Dynamic behaviour of a floating water droplet with hydrocarbon-degrading bacteria

This chapter will discuss the effect of bacteria on contact angle and also the volume of the droplet floating on oil surface. Moreover, the applicability of this new method for addressing the oil spills problem will be discussed.

4.1. Results and discussion

4.1.1. General observation

The oil and solution densities were 0.83 and 1.041 g/cm^3, respectively. The droplet containing bacteria was released on the oil/air surface as before. The water droplet behaved differently depending on the deposition volume. For small volume, < 0.5 µl, the droplet did not break and remained floating as a whole (Figure 4-1). Hereafter, it is referred to as the primary droplet. In contrast, the larger deposition volume, e.g. greater than 0.8 µl, resulted in the breaking up of the droplet. In these instances, the droplet was broken into two parts: one sunk to the oil layer and the other floated on the oil surface. The leftover, denoted at secondary droplet, remained floating sufficiently for the bio-decomposing process to complete. Effectively, the maximum floatable volume was ~ 0.3 µl, which was 20 vol% of the non-bacterial droplet (20). The trends of contact radius and contact angle in the primary and secondary floating droplets were analyzed and discussed below.

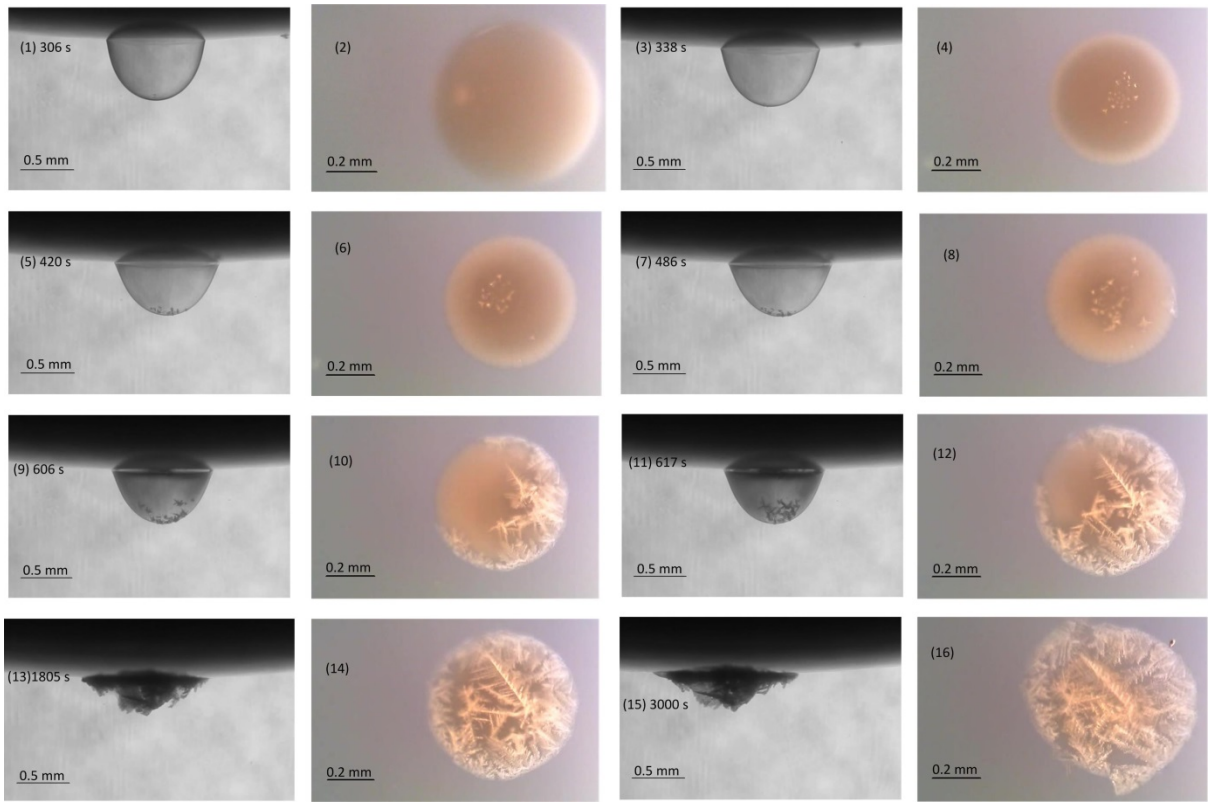


Figure 4-1. General observation of a floating water droplet containing bacteria, SDS, enzyme and NaCl on paraffin oil. The droplet shows the primary droplet which does not break and remains floating on the oil surface. Two types of images shows the side and top of the droplet. Side view is important for analysis, so it has higher/clearer magnification.

4.1.2. Primary droplet

Initially, the droplet spread out at the beginning and then formed a droplet shape with contact angle ~ 50^{o}. The oil/water interface was initially dark, which may indicate a saturated surfactant layer. The droplet color became gradually clearer as the bacteria grew till it became colorless (Figure 4-2-11). The solid biomass appeared in the oil/water interface after ~ 15 minutes and dominated the oil/water interface (Figure 4-2-20). Subsequently, the droplet interface was no longer governed by the Young-Laplace equation. Eventually, the water content was consumed by bioprocess and/or evaporation (Figure 4-2-21).

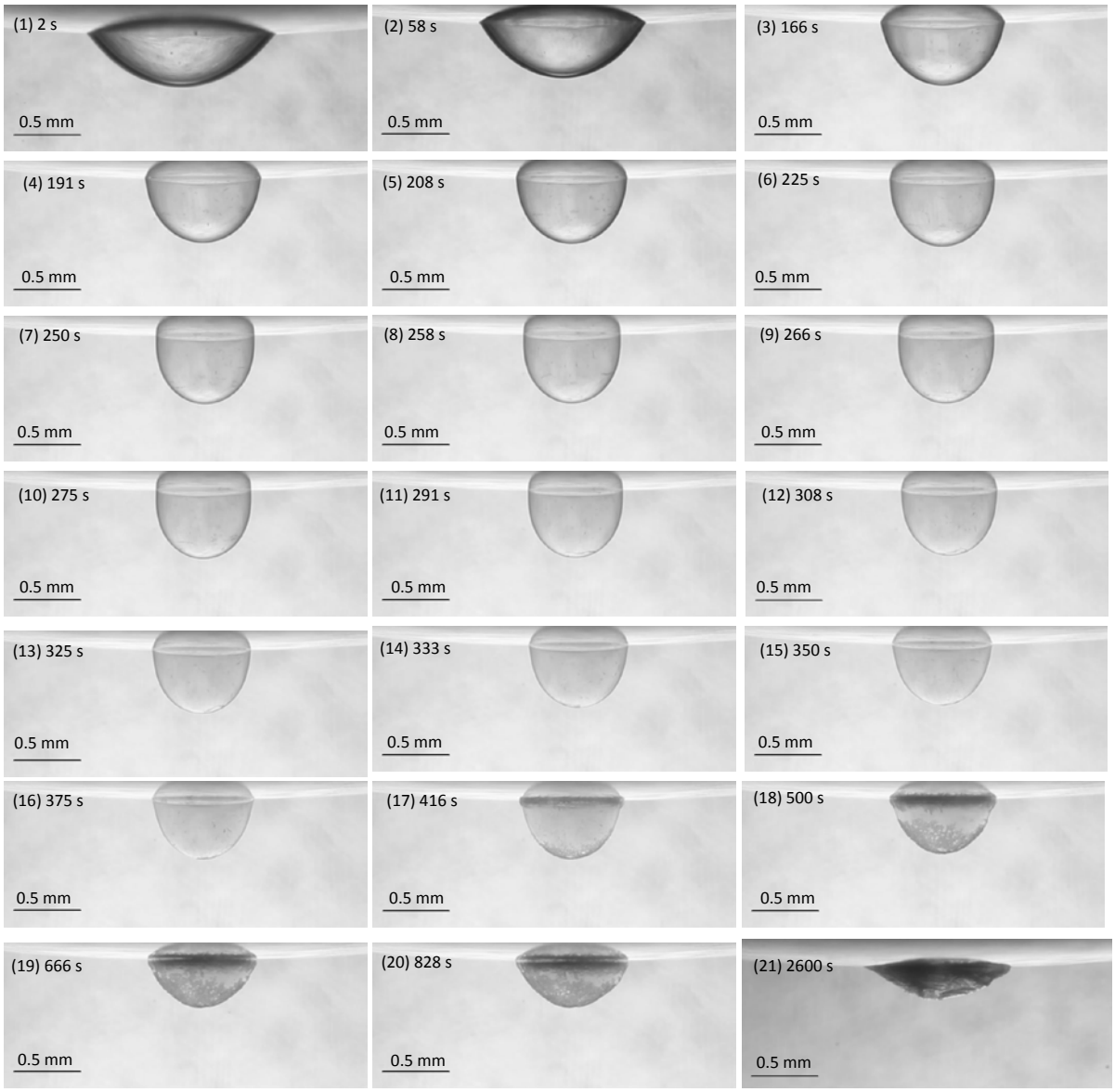


Figure 4-2. Droplet images after deposition of a primary droplet (Volume ~ 0.3 µl).

The shape of the droplet was quantified in term of contact angle and contact radius (Figure 4-3). Contrary to droplet without bacteria in the previous study (20), in which the contact angle change monotonically with time, the contact angle of this droplet increased and then decreased. The change in droplet behaviour was due to the biological process, which will be discussed below. It should be noted that after 8 minutes, the edge was no longer sharp due to the growth of bacteria at the air/water/oil contact line. The bacteria growth can be visually observed in Figure 4-2-17. As the result, the contact angle in Figure 4-3-a became scatter after 8 minutes.

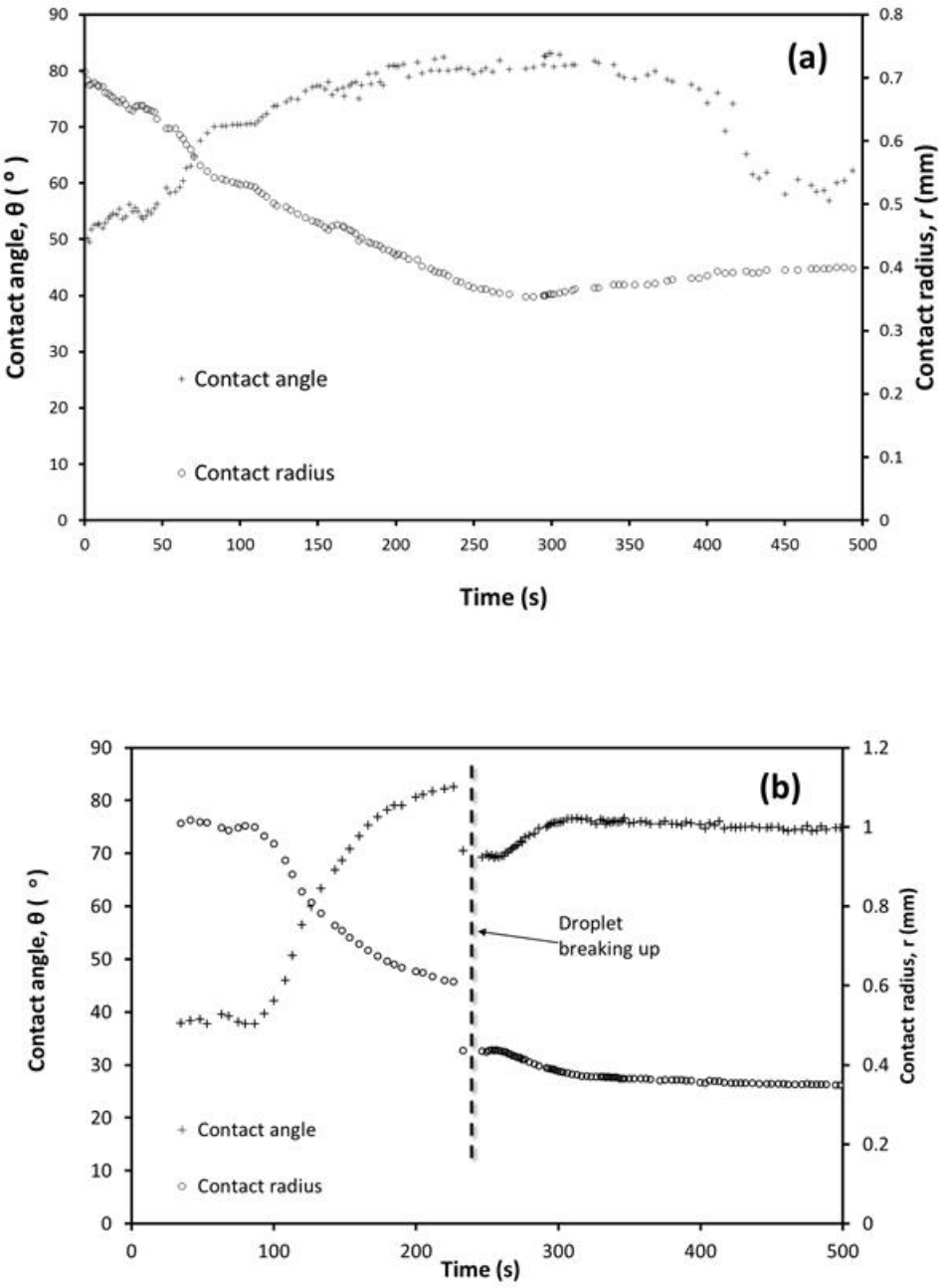


Figure 4-3. Transient contact angle and contact radius after deposition: (a) ~ 0.3 µl and (b) initial volume (before breaking up) ~ 0.85 µl.

4.1.3. Secondary droplet

For larger volumes, the water droplet broke into two parts, typical within 5 minutes. After deposition, the droplet spread out to form a shape similar to that of the primary droplet, before breaking up at a contact angle ~ 90^{o} (Figure 4-4). The contact angle increased from 41° (Figure 4-4-1) to 80^{o} (Figure 4-4-3). Figure 4-4-4 shows the deformation and separation of the droplet. As shown previously (20), the maximum floatable volume varies with the combinations of surface tension. In this study, the bacteria/nutrient mixture produced different surface tensions and limited the floating volume.

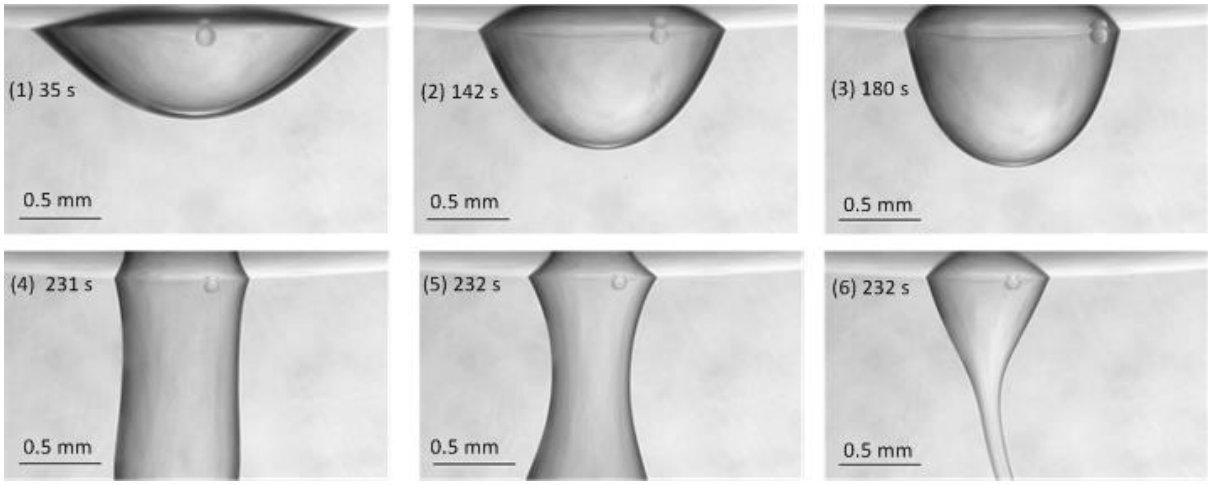


Figure 4-4. Breaking up the floating droplet (initial volume ~ 0.85 µl)

After breaking up, a secondary droplet remained floating with the contact angle of approximately 70^{o}. The remained volume was typically ~ 0.1 µl. The bio-activity was visible within the droplet. The biomass emerged to disrupt the oil/water interface as with primary droplet. Eventually, the water content inside the droplet was completely removed. The contact angle increased sharply before the breaking (Figure 4-3-b), which corresponded to the reduction in oil/water interfacial tension (20, 130). After breaking, however, the contact angle changed slightly. Details of the analysis can be found in Appendix A1.

4.1.4. Volume evaluation

As the experiment progressed, the droplet volumes were calculated and presented in Figure 4-5. The volume of bacteria-containing droplets initially decreased at a similar rate with non-bacterial droplets. The linear relationship indicated a constant rate of evaporation. From the slope of volume reduction (straight lines the insert), the evaporation rate was estimated using the following equation:

$$e = \frac{dV}{dt}\frac{\rho}{A} \qquad \text{Equation 4-1}$$

Where e is the evaporation rate g/cm^{2}s, V is the droplet volume (cm^3), A is the cross-section area of water/air interface (cm^2), ρ is solution density (g/cm^3)

From the above equation and slope (dV/dt), the evaporation rate was estimated ~ 6×10^{-5} g/cm^{2}s, which was in the same order with the evaporation through of a surfactant-covered air/water interface (2×10^{-5} g/cm^{2}s) (121).

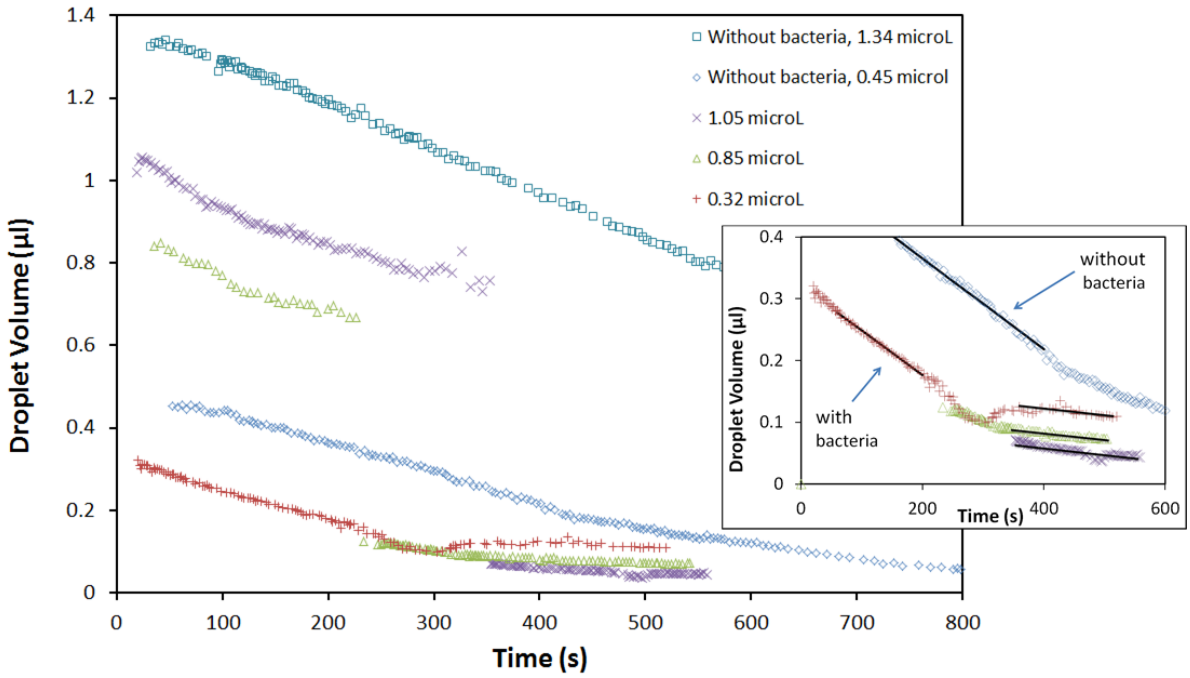


Figure 4-5. Droplet volume without and with bacteria. Lines represent linear regression of volume reduction.

The volume reduction however altered drastically for bacteria-containing droplets after ~ 5 minutes. For the primary droplet, the volume became almost constant (shown in insert). The change in volume was also confirmed by the contact radius in Figure 4-3-a. For the secondary droplets, the volume also became plateau after breaking up. The moderate reduction in evaporation indicated that a bio-produced film has been formed on the air/water interface.

In addition to biofilm formation at the air/water interface, biodegradation process may also have increased the droplet volume by producing water and water-soluble organics. The biodegradation of hydrocarbons usually consists of two steps: functionalization of hydrocarbon and bio-growth (14, 60). In the first step, alcohol, aldehyde, and fatty acids are produced. Consequently, these intermediate products are converted to carbon dioxide, biomass and metabolites in the second step. The overall process of the first step can be demonstrated as:

$$C_xH_yCH_3 \rightarrow C_xH_yCH_2OH \rightarrow C_xH_yCHO \rightarrow C_xH_yCOOH \qquad \text{Equation 4-2}$$

In this step, the bacteria play a catalytic role to oxidize alkane as summarized in the chemical equation below:

$$C_xH_yCH_3 + \frac{3}{2}O_2 \xrightarrow{Bacteria} C_xH_yCOOH + H_2O \qquad \text{Equation 4-3}$$

As the result, the bio-processes increased the droplet volume by producing water molecules and soluble organics. It should be noted that fatty acids and alcohols are also surface active, and thus can emulsify and dissolve hydrocarbons into the water droplet. The production of these soluble organics in combination with bio-film formation effectively kept the droplet volume constant as shown in Figure 4-5. In the second step of biodegradation process, the buildup of biomass expectedly required water.

It is noteworthy that the oil/water and air/water surface tensions only influenced the droplet shape in the first 5 minutes, as shown in Figure 4-3-a and 3b. Previous studies have shown that

the bio-film reached equilibrium for air/water (113) and oil/water (131, 132) interfaces within few minutes and few hours, respectively. Hence, the reshaping process was apparently attributed to the biofilm on air/water interface only. In contrast, the oil/water interfacial tension remained dominated by SDS, a strong anionic surfactant. The shape of the droplet remained virtually unchanged during the later stage, which demonstrated a minimal impact of bio-activities on the interfaces. It can be concluded that once the bio-film is established on air/water interface, the bio-activities have a negligible impact on both interfacial tensions and floating behaviour.

4.2. Summary

The study quantified the influences of bio-processes on the dynamic behaviour of a floating water droplet on oil layer. It was found that the maximum floatable volume was 0.3 µl, which is only 20 vol% of the surfactant-stabilized droplet. However, the bacteria-containing droplets remained floating sufficiently to complete the bio-processes. The formation of a biofilm on the air/water interface, which is a key characteristic of the bacteria-containing floating droplet, significantly shaped the droplet behaviour by regulating the evaporation and air/water interfacial tension. However, the dynamic influence reached equilibrium in 5 minutes. Afterward, the bio-activities had an insignificant impact on the droplet floatability. The water droplet was eventually consumed by the bacterial growth.

From the results, there are a number of important insights for application: (i) the droplets should be reapplied every 20 minutes to optimize the biodegradation; (ii) the droplets should be applied by spraying devices with precise size control, < 0.3 µL; (iii) the floatability of the droplet on specific oils can be determined after the first 5 minutes.

From the droplet size, the optimal application rate can be estimated as approximately 0.3 liter of solution per square meter of oil spills. However, the bacterial solution in this study can be

prepared using seawater with dry bio-materials and surfactant. The study demonstrated that the new method can effectively degrade the oil layer from the aqueous environment. The products, biomass or fatty acids, are far less toxic than the original oils. Although a complete removal is not possible, the method can be applied to significantly reduce the oil layer, before dispersing. Depending on the location of the oil spills, it may take days to prepare/transport the dispersant to the site. Consequently, there is a window opportunity to apply the method to complement the current practices. A partial decomposition will reduce the amount of required dispersants and the overall environmental impact of oil spills.

In actual oil spill, waves, tidal streams and currents, heavy oil with complex structure are the factors that have high impacts on the results. Emulsification of water droplet containing bacteria is one of the consequences of windy weather with big waves. In this condition, the contact between oil and bacteria is increased and as a result enhance the degradation of oil even though the droplets are not floatable. Also, temperature can influence the result by (i) increase the bacterial activities, and (ii) increase the evaporation rate. One is favourable and the other one is limitation factor respectively.

Further studies with the focus on application of this method with different material and different oil contaminants were conducted which is present in the next chapter in detail.

Chapter 5: The floatability and behaviour of a floating water droplet in different conditions

This chapter verifies a floating water droplet in the bio-decomposition with different systems. Hence, the study in the previous Chapter is extended to different systems to understand the influence of the following factors: (i) nutrient medium, (ii) surfactant and (iii) oil. These variations require verifying different factors in the real application. Hence, the nutrient, surfactant and oil were varied while the aqueous solution was prepared with the same bacteria concentration.

5.1. System selection

To understand the influence of the materials on the systems, three new systems were selected. First, MIA solution was used to replace enzyme as the nutrient sources. Second, triton-705 was used to replace SDS as the stabilizing surfactant. Finally, diesel was used instead of paraffin oil in the last system. The last system of this study is related to verification of this new method in the degradation of different oil particularly the oil with resistance to biodegradation. The reason for preparing the solution separately is to be comparable with the results in chapter 4. Lists of materials were used in the three systems are tabulated in Table 5-1.

Table 5-1. Lists of the materials

Name	grade	supplier
Sodium dodecyl sulfate (SDS)	99	Sigma-Aldrich
Triton-705	99	Sigma-Aldrich
NaCl	99	Sigma-Aldrich
Paraffin oil		Digger Inc., Australia
Diesel oil		BP Australia
Enzyme		Enzyme Wizard PTY LTD
MLA Medium		As noted
Bacteria		Solution Unlimited Australia

5.2. Results and discussion

Three sets of experiment were developed. The first two studies were conducted on paraffin oil while the third was on diesel oil. The study on paraffin oil considered the impact of surfactant and nutrient variations in the same environment. However, the study on diesel oil verified the floatability of a water droplet on different hydrocarbons.

5.2.1. MLA medium on paraffin oil (System 1)

The effectiveness of this new method for degradation of oil spillages was performed by the combination of bacteria, SDS and NaCl solution in the presence of MLA medium instead of Enzyme. As shown in Figure 5-1, the water droplet did not break even though the volume of water droplet is approximately five times bigger than the volume of a water droplet in chapter 4. Contact angle was approximately 70°, and after 30 minutes, it decreased to 55° (shown in Figure 5-2). Details of the analysis can be found in Appendix A2.

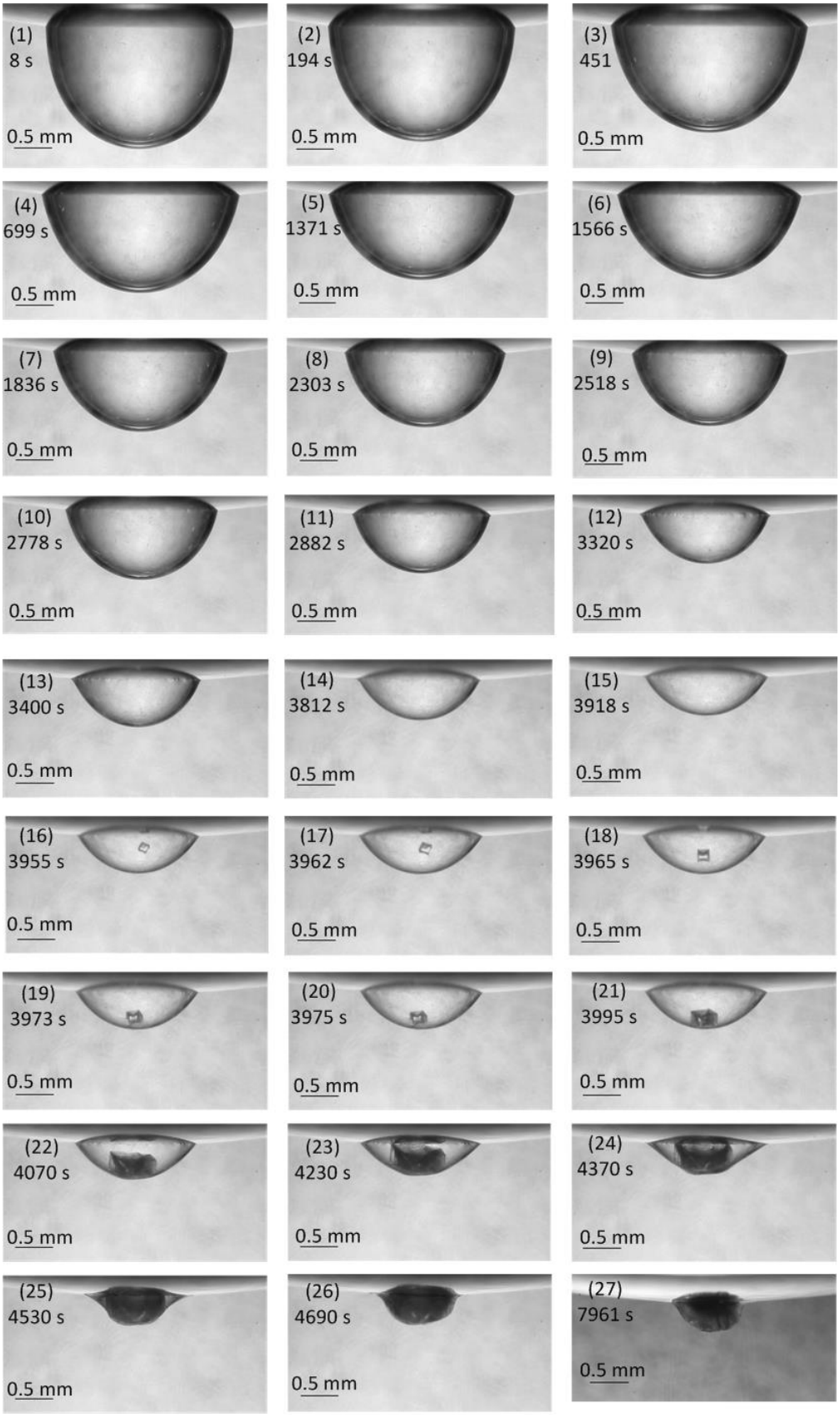


Figure 5-1. Water droplet shapes containing SDS, NaCl, Bacteria and MLA medium in paraffin oil.

The water droplet is more stable compared with the droplet containing enzyme since the density of water droplet in the presence of MLA medium (1.021 gr/cm^3) is less than the previous study (1.044 gr/cm^3). However, this medium is not suitable for bacteria because biofilm formation is started after one hour (Figure 5-1-16). Bacteria in the presence of enzyme are more active as biofilm formation can be observed after approximately five minutes.

The solid objects in the droplet may/may not be biofilms. In the previous study, biofilm formation was stated in the air/water interface because bacteria may have enough oxygen for biodegradation. However, in this study, biofilm or solid objects was observed in the droplet (Figure 5-1-17), and after being heavier tend to deposit at the bottom of the droplet. Hence, these solid objects can be small salt crystals generating as a result of salting out phenomena (112). This crystallization or biofilm formation increased, and the interface was solidated which can be observed in Figure 5-1-26 and Figure 5-1-27.

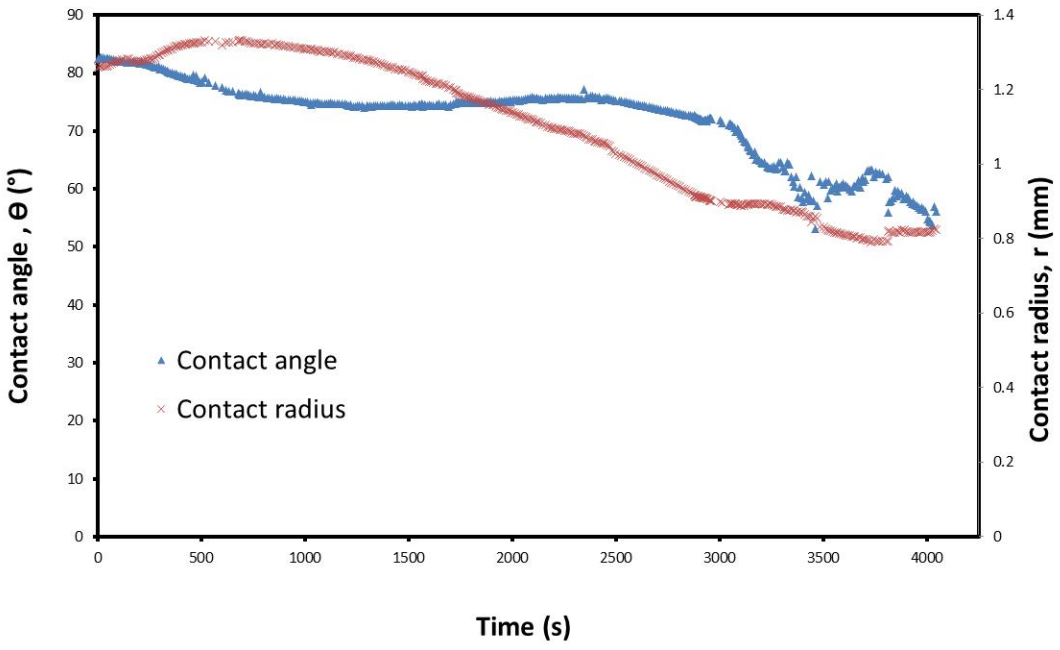


Figure 5-2. Transient contact angle and contact radius after deposition (initial volume ~ 5µl)

As the experiment progressed, the droplet volumes were calculated and presented in Figure 5-3. The volume of the droplet decreased with the constants slope in the whole process.

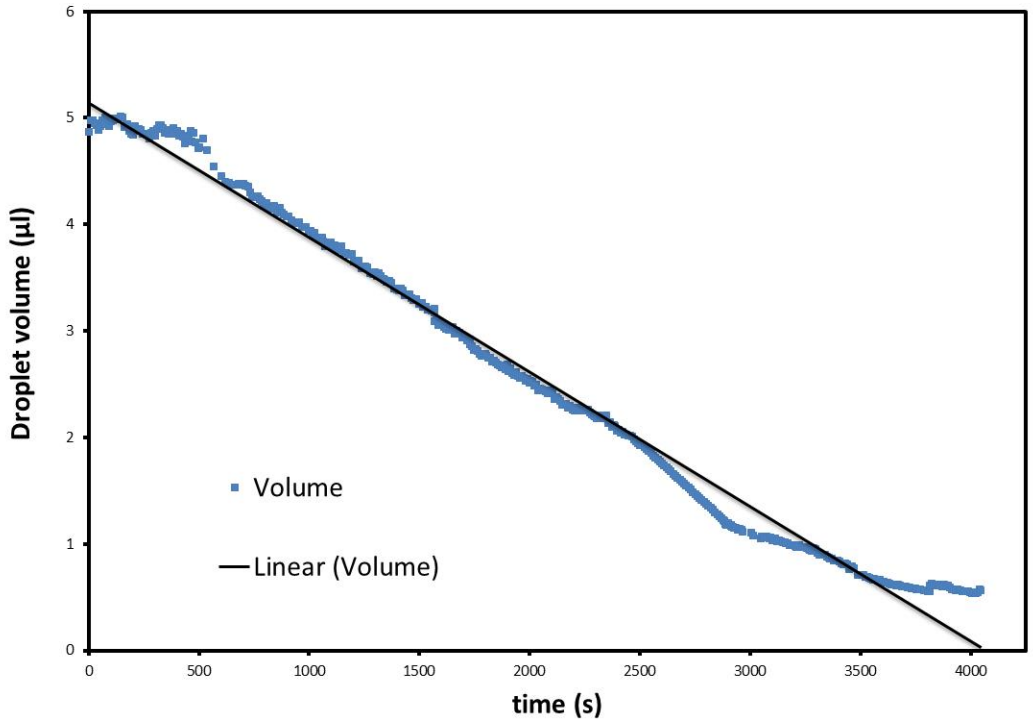


Figure 5-3. Volume of the droplet containing MLA medium (initial volume ~ 5µl)

The evaporation rate which is 2.8×10^{-5} g/cm^{2}s was obtained from the Equation 4-1. The rate of evaporation is less than the droplet without bacteria which may be justified by the chemical interaction between surfactant, medium, NaCl, paraffin oil and bacteria (Table **5-2**).

Table 5-2. Evaporation rate in the presence of bacteria and without bacteria

Solution	Volume (µl)	Evaporation rate (g/cm^2s)
Droplet (water, NaCl, SDS)	0.5	5.3×10^{-5}
	1.4	5.3×10^{-5}
Droplet (MLA medium, water, bacteria, NaCl, SDS)	5	2.8×10^{-5}

In comparison with the previous Chapter, volume reduction rate was insignificant for the whole of the process. This can be due to the weak effect of MLA medium on bacteria growth. In this system, volume increment as a result of the generation of intermediate product in the bioprocess was not observed. Hence, these observations indicated that MLA medium is not suitable for growth of bacteria, and, as a result, cannot stimulate them to decompose paraffin oil.

5.2.2. Non-ionic surfactant droplet on paraffin oil (System 2)

Surfactants support the floatation of water droplets on oil surface and increase the stability of water droplets (20). In this part of the study, Triton X-705 which is a nonionic surfactant was employed to verify the effect of different surfactants in floatability of water droplets on oil surfaces and bacterial activities.

Shape and behaviour of floating water droplet are similar to the secondary droplet in chapter 4. The contact angle of deposited droplet increased from 41° (Figure 5-4-1) to 84° (Figure 5-4-4), and radius decreased.

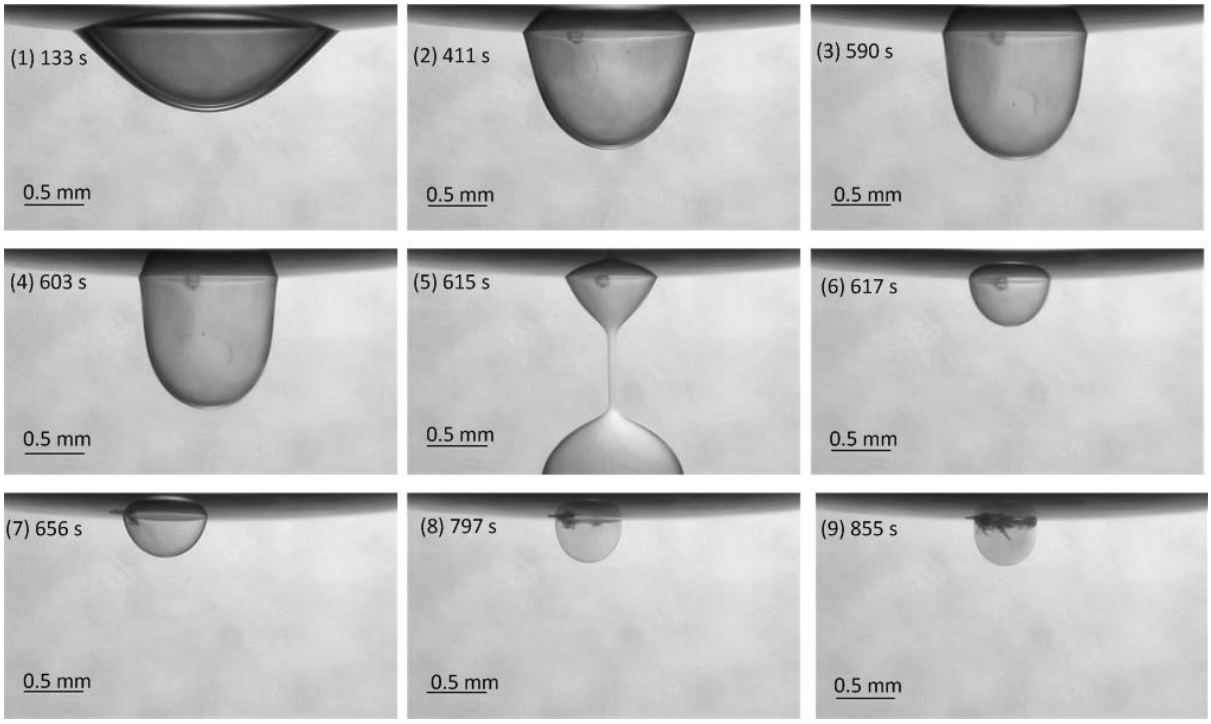


Figure 5-4. Water droplet containing Triton, NaCl, Enzyme and bacteria on paraffin oil surface.

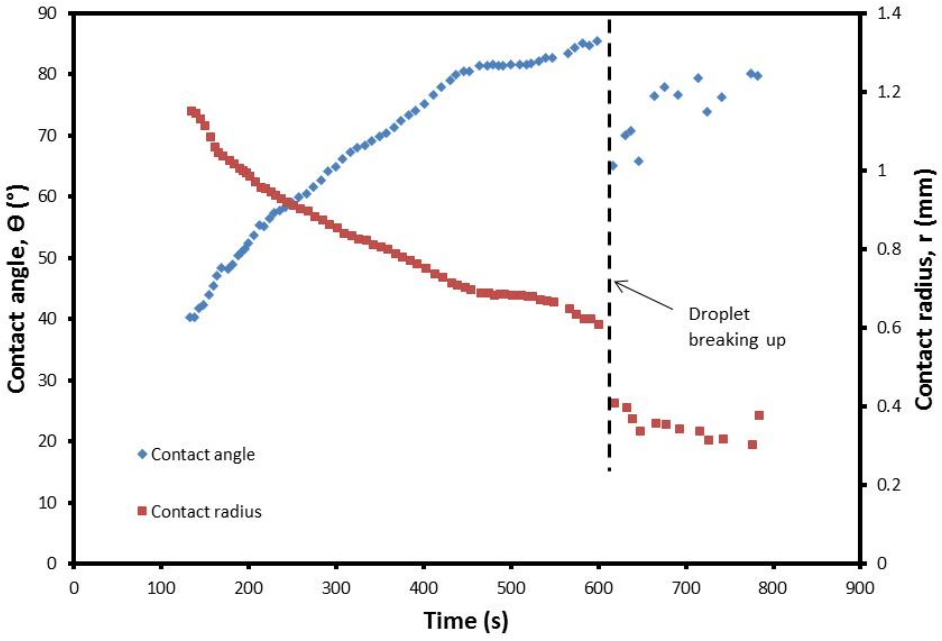


Figure 5-5. Transient contact angle and contact radius (initial volume ~1.4 µl)

The volume of deposited oil was ~ 1.4 µl, which was higher than the maximum volume of floating water droplet (~ 0.3 µl) described in Chapter 4. Consequently, it broke up after ~7 minutes. The volume of remained droplet was 0.09 µl as shown in Figure 5-6. The rate of evaporation of the droplet (1.1×10^{-5} gr/cm^{2}s) is less than the droplet without bacteria (5.3×10^{5} g/cm^{2}s) which can be described by the presence of bacteria and effect of intermediate products such as alcohols and surfactants, and also biofilm formation in the air/ water interface.

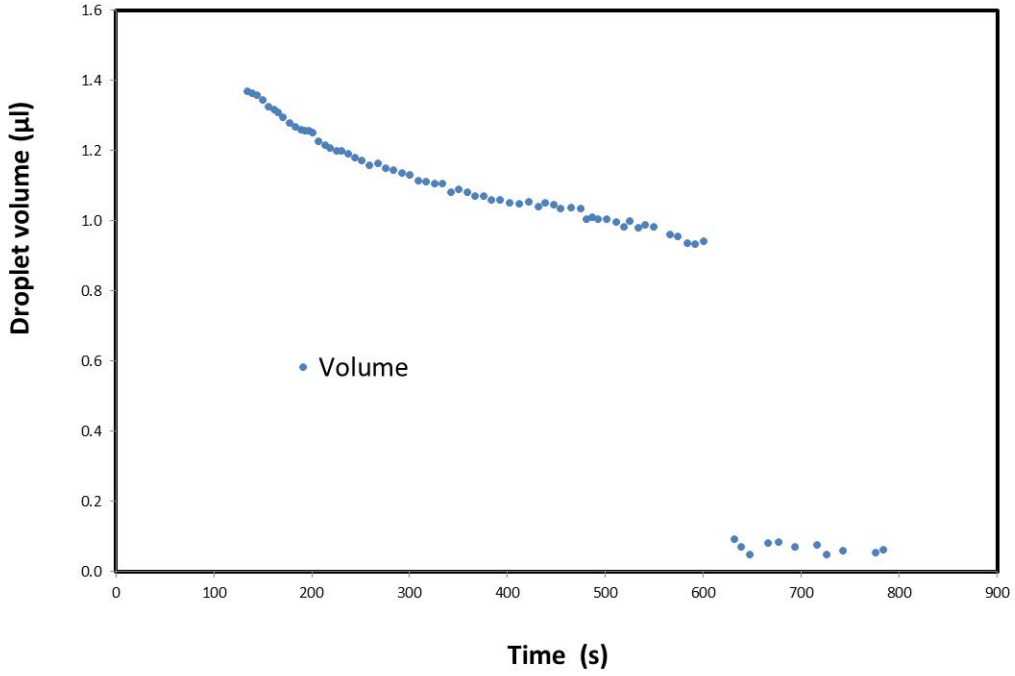


Figure 5-6. Droplet volume containing Triton, NaCl, enzyme, bacteria in paraffin oil surface

The results of this study are consistent with the previous study in terms of contact angle, radius, and volume changes. Therefore, anionic surfactants and non-ionic surfactants have an identical role in floatability of the water droplet and also bacterial activities.

5.2.3. Floating droplet on diesel oil (System 3)

Water droplet containing SDS, NaCl, enzyme and bacteria was deposited in diesel oil surface. Similar to the droplet in paraffin oil, this droplet spread out at the beginning, and after first

breaking up, remained droplet was analysed as described in chapter 3 to find contact angle, radius and volume.

Contact angle decreased from ~ 62° to ~ 55° at the beginning (Figure 5-8) and then increased until the second breaking up accrued (Figure 5-7). The remained droplet repeated the similar behaviour. The contact angle of remained droplet decreased and then increased until the last breaking up accrued.

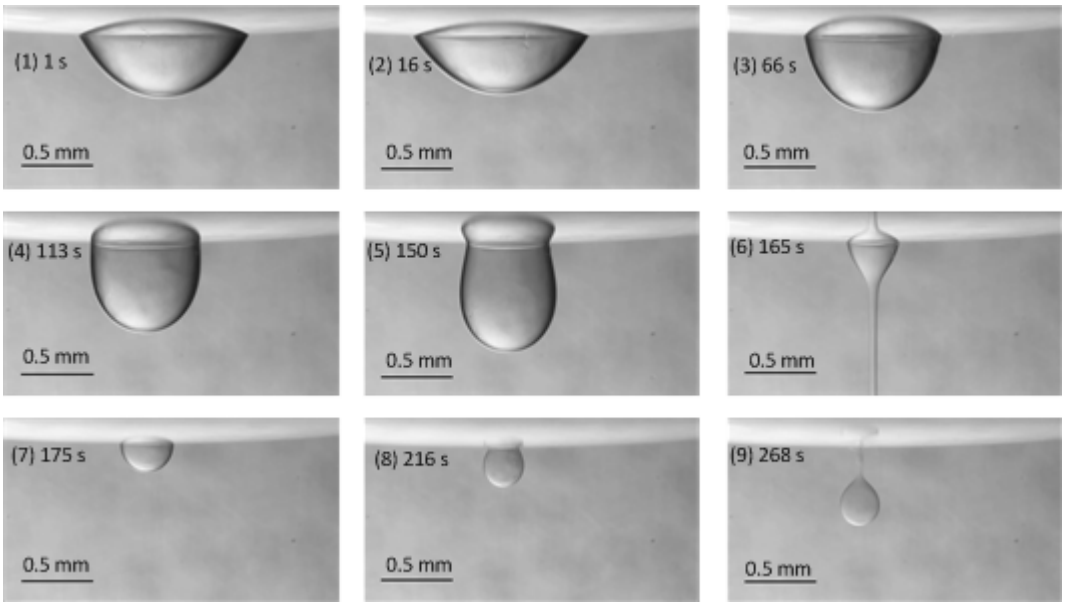


Figure 5-7. Deposition of droplet containing bacteria, enzyme, SDS, NaCl in diesel oil (initial volume of ~ 0.22µl)

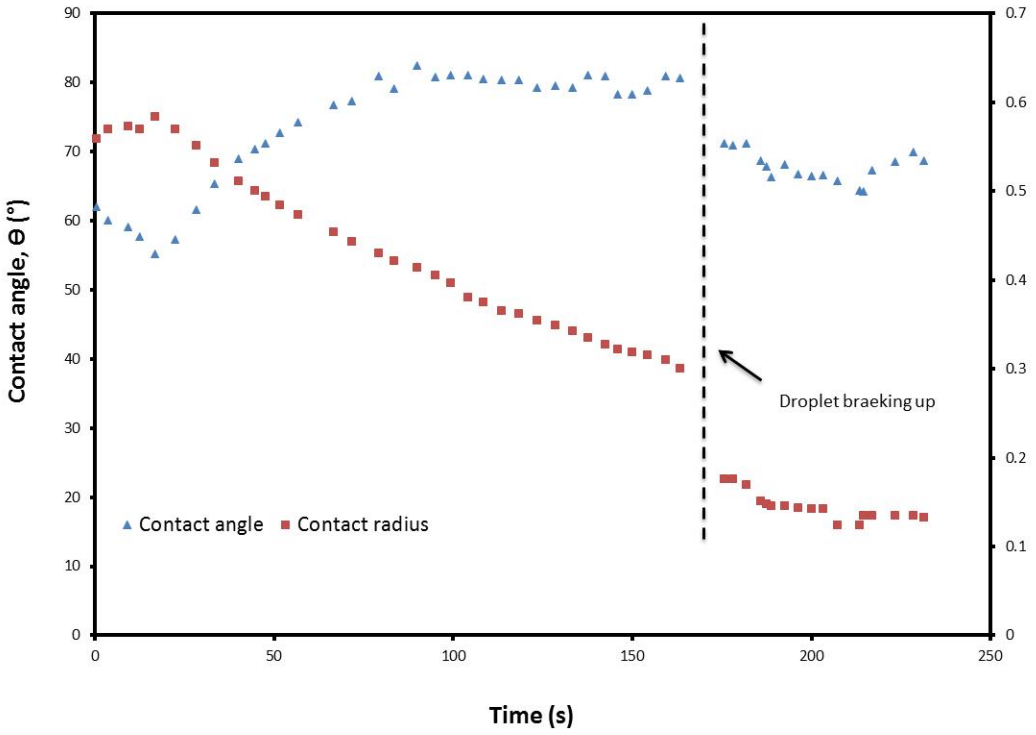


Figure 5-8. Transient contact angle and contact radius (initial volume ~ 0.22µl)

This experiment was repeated with different droplet volume. After deposition of each droplet, contact angle increased, and contact radius decreased (Figure 5-9, Figure 5-10, Figure 5-11).

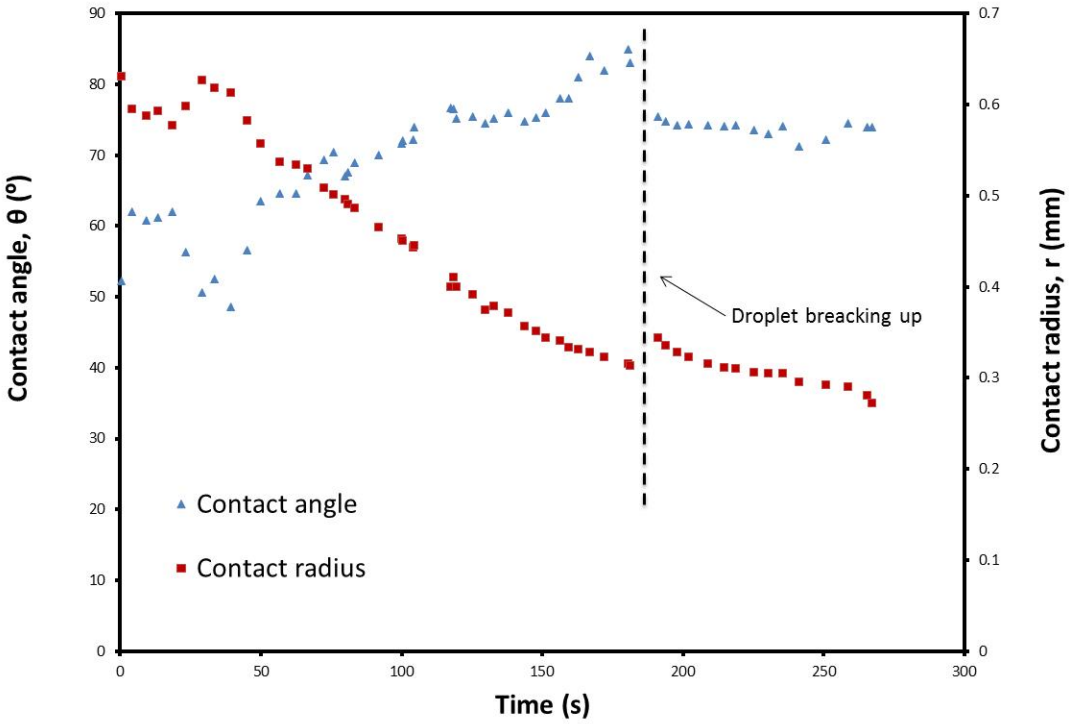


Figure 5-9. Transient contact angle and contact radius (initial volume ~ 0.24µl)

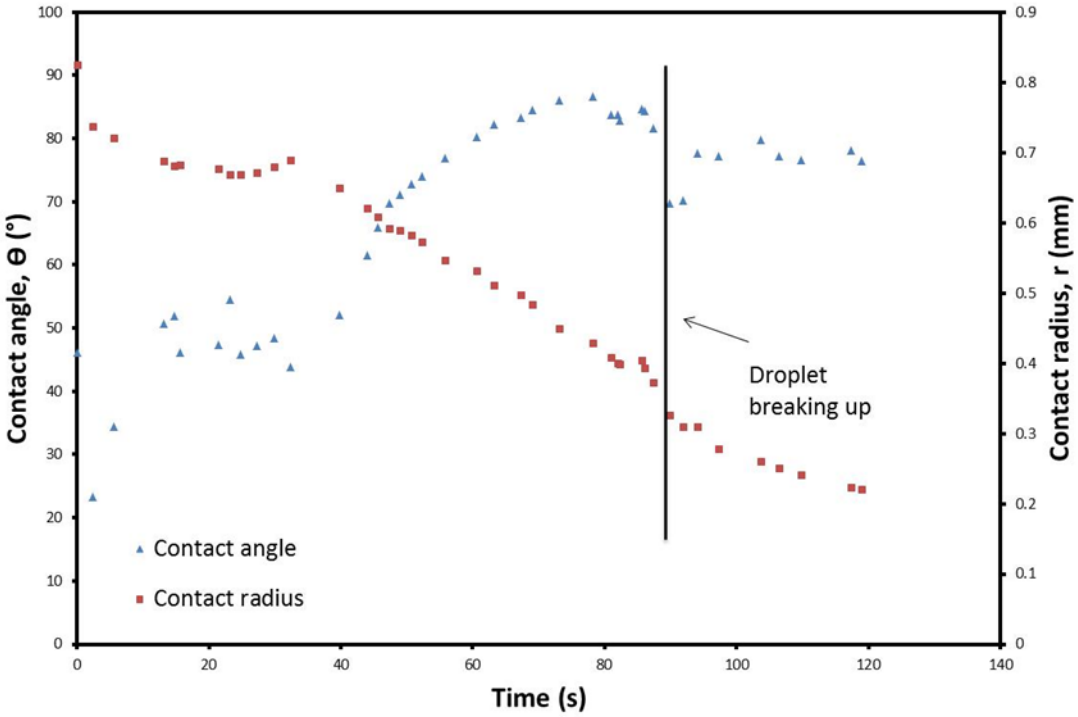


Figure 5-10. Transient contact angle and contact radius (initial volume ~ 0.4 µl)

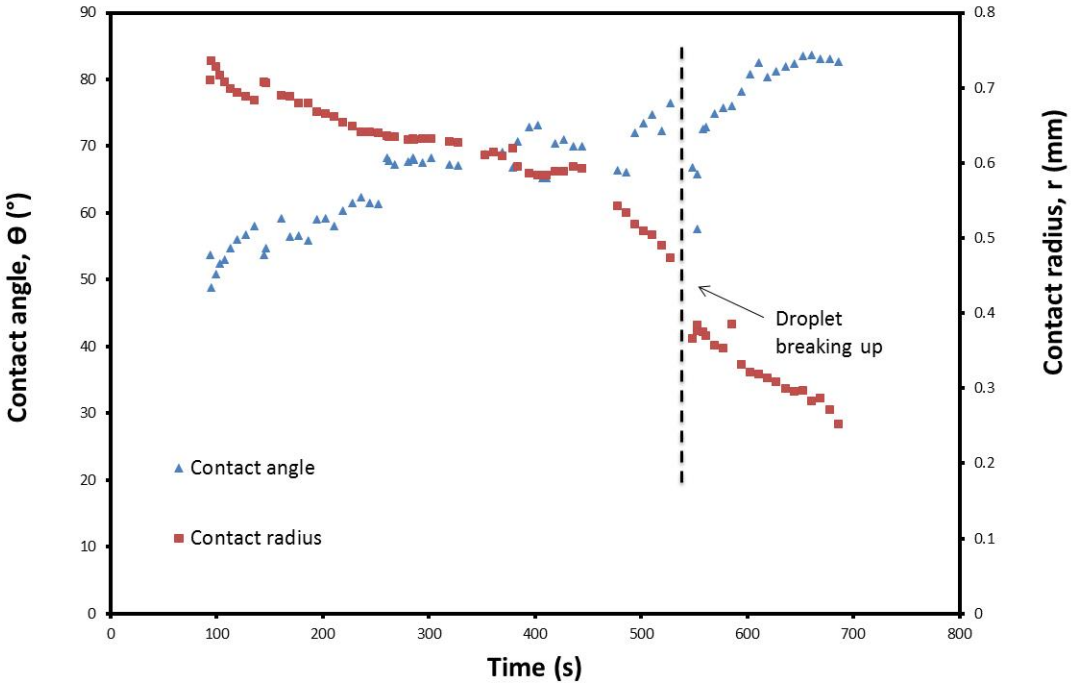


Figure 5-11.Transient contact angle and contact radius (initial volume ~ 0.45 µl)

Contact angle and radius changes in the droplet floating in diesel oil are similar to the droplet in chapter 4, but the only difference is related to floatation time of the droplet. Water droplets in diesel oil were not stable (Figure 5-7), and they mostly sank after two or three times breaking up even though the density of paraffin oil and diesel are virtually similar (Table 3-2). Moreover, this behaviour is not related to the volume of droplet since different volume droplets were deposited in diesel oil and all have the similar behaviour. This can be due to the diesel/ water interfacial phenomena.

Few droplets in diesel oil remained floating, and bioactivities and biofilm formation can be observed on those droplets floating on the diesel oil surfaces (Figure 5-12).

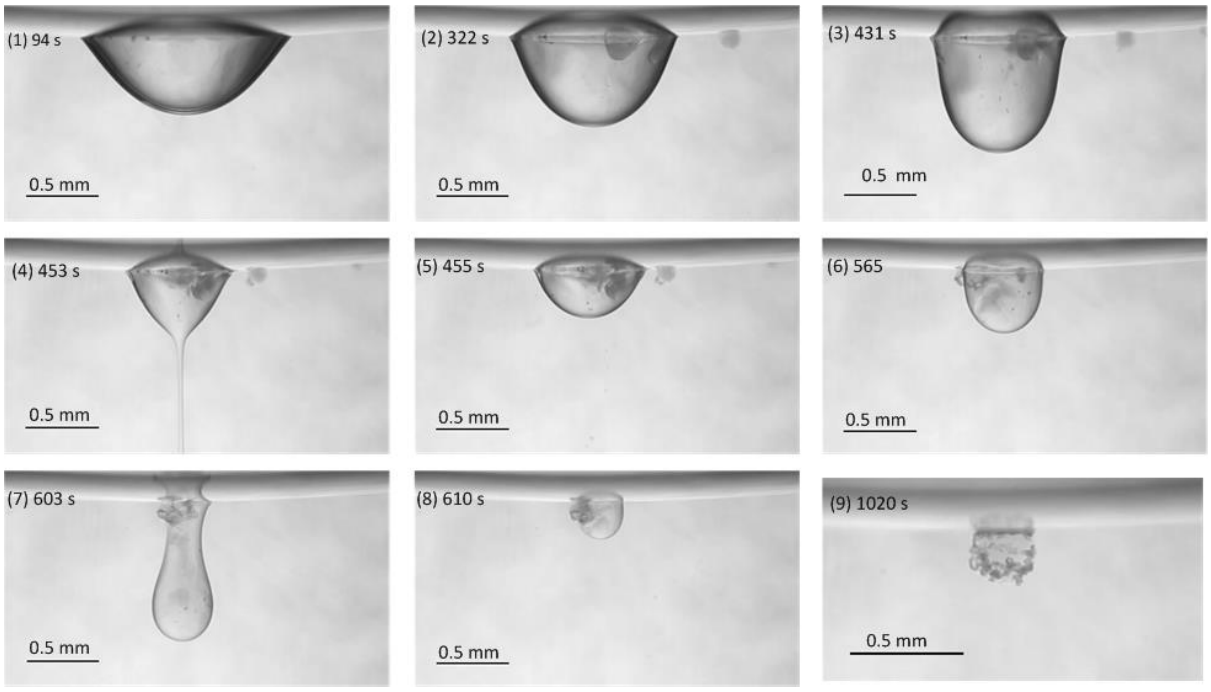


Figure 5-12. Transient contact angle and contact radius (initial volume ~ 0.45 µl)

As shown in Figure 5-13, effect of biofilm and bioprocess can be evidently observed in the droplet volume.

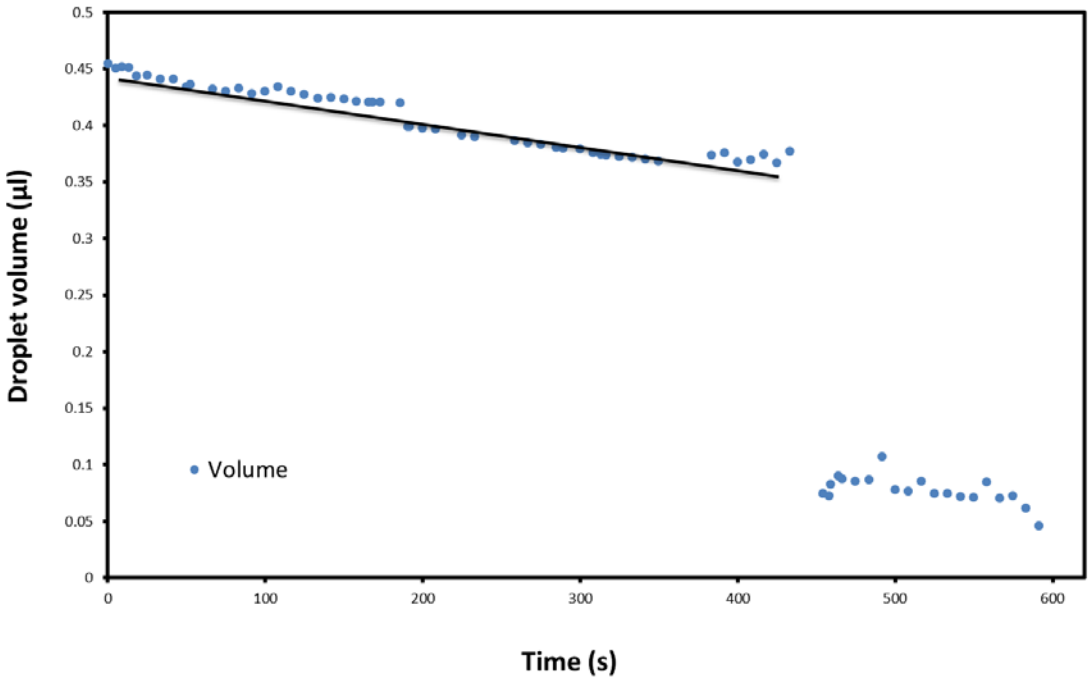


Figure 5-13. Volume droplet with biofilm, initial volume ~ 0.45 µl

The evaporation rate of the droplet with a volume of 0.45 µl with biofilm formation is 1.18×10^{-5} gr/cm^{2}s which is less than the non-bacterial droplet (6×10^{-5} gr/cm^{2}s). Bioprocess, intermediate products, and biofilm can be the most significant reason for these differences on evaporation rate. While the formation of biofilm can be partially responsible for the reduced evaporation, the chemical composition of diesel could the main factor. Since diesel contains lighter hydrocarbons with small but finite solubility, these compounds may be dissolved into air/water surface of the floating droplet. The complete wetting of thin oil film on water surface has been well documented (133) . Such layer can quickly reduce the evaporation rate. The images in Figure 5-12 also confirm the phenomena: biofilm tends to grow on the oil/water interface rather than the air/water interface.

The influence on evaporation can be the most significant difference between diesel and heavier oil. The reduced evaporation will allow longer stability of the droplet and increase the biodegradation. On the other hand, the formation of the thin oil layer might reduce the oxygen diffusion into the droplet and encourage anaerobic, instead of aerobic, bio-processes.

In summary, the droplet behaviour on diesel oil was fundamentally different to paraffin oil. Diesel consists for smaller hydrocarbons which can be soluble, to a limited extent, in water.

Overall, the floating on diesel is harder than in paraffin oil. Consequently, the method is more favorable to heavier hydrocarbons.

5.3. Summary

The experimental results showed that floatation of water droplet containing bacteria are changed by the medium, surfactant, and the oily environment. Results can be resumed in Table 5-3.

Table 5-3. Results obtained under different conditions

Solution	Breaking up	Evaporation rate (gr/cm^2s)	Droplet volume (µl)	Air/water surface tension (mN/m)	Density (gr/cm^3)
Droplet without bacteria	No	5.3×10^{-5}	0.5, 1.4	26.52	1.0224
System 1	No	2.8×10^{-5}	5	26.41	1.0215
System 2	Yes	1.1×10^{-5}	1.4	26.58	1.0458
System 3	Yes	1.2×10^{-5}	0.45	25.5	1.0442

The experimental results showed that anionic and non-ionic surfactants have the same effect on floatability of water droplets in paraffin oil in the presence of bacteria, enzyme, and NaCl. By changing the nutrient source of bacteria to MLA medium, droplets containing this medium did not break after deposition, and remained stable on oil surface. However, the MLA medium was apparently ineffective nutrient source for hydrocarbon-consuming bacteria. As a result, the floatation of water droplets in oil surfaces did not change and the system remained static for a long time (Figure 5-3).

Diesel oil as a complex hydrocarbon compounds used in this study. Paraffin oil was replaced with diesel oil to verify the effect of different oil on bacterial activities. It was found that the shape of droplets and changes of contact angle and radius are similar to droplet deposited on paraffin oil. However, droplets were not stable on diesel surface and in most cases the droplet sank. The bioactivities and biofilm formation were observed in the remained droplets.

The evaporation rate varied significantly from one system to another. This indicated that the bio-film on air/water interfaces was not constant. Instead, the nutrients, surfactants and the environment can significantly influence of the evaporation. Consequently, any potential application of the method should be selected specifically for the local conditions.

Chapter 6: Conclusions and Recommendations

6.1. Conclusions

6.1.1. Influence of bacteria in floatability of water droplet

The objective of this study was verification of bacterial effects on floatability of water droplets with SDS, enzyme and NaCl on paraffin oil. For this study, SDS was used to support the floating droplet. The enzyme also was employed to be the nutrient source for the growth of bacteria.

One of the outcomes of this project was floatation of water droplets on oil surface. The droplets remained floating with volume of 0.3 µl. A droplet broke up with the volume of more than 0.3 µl, but the remained droplet was stable on oil surface. Furthermore, completion of bioprocess was observed in all droplets due to formation of biofilm, which is the result of biodegradation.

It was also found that biofilm in air/ water interface affected on evaporation rate of the water droplet. The volume of droplets did not change and remained constant after generation of intermediate products and biofilm. Finally, water was consumed totally by bacteria.

Therefore, the experimental results showed that the decomposition processes by bacterial activities can significantly affect the droplet shape and droplet behaviour in terms of contact angle, radius, and volume.

6.1.2. Effectivity of floating water droplet with different materials and environment

The second purpose of this study was the determination of behaviour of floating water droplet in the presence of different materials. Three sets of experiments were conducted by changing surfactant and medium and the environment.

The droplet containing MLA medium was stable for more than one hour on the paraffin oil, but biofilm was not observed due to the ineffectiveness of this medium for growth of bacteria.

A water droplet, containing Triton-705, showed a similar effect as with SDS-containing droplet. Hence, it can be concluded that the nature of the surfactant, e.g. nonionic versus anionic, has a minimal influence on the droplet floatability and bio-processes.

The last part of this study focused on the effectivity of floating water droplet in the decomposition of diesel oil. The results indicate that the initial shape and behaviour of droplet containing SDS, enzyme, NaCl and bacteria are similar to the droplet floating on paraffin oil. However, the droplet on diesel oil is not stable and tends to sink very quickly.

6.2. Recommendations for future work

This project verified the influence of bacteria on floating water droplets on oil surfaces such as paraffin oil and diesel oil. A fitting-edge model was developed to calculate the contact angles between the water droplets and interfaces involved. Effect of different material was also investigated in floatability and shape of the floating water droplets. Some recommendations for future work are provided bellow.

It is recommended that for future investigations, asphalthenes, which is very difficult to decompose because of its complex arrangement of hundreds of benzene rings, is used and verified the water/oil interface.

In this study, NaCl was used in all the experiments. Since other inorganic ions such as Mg^{+2}, Ca^{+2}, $(SO_4)^{-2}$ are naturally found in the environment, the experiments should be conducted with different salts to verify the effect of salt on the process.

Biofilms, which is one of a factor in regulating the evaporation, should also be examined further. The experiment can be performed in different temperature and nutrients to verify the

influence of these environmental variable to biofilm development and consequently in floatability of water droplets. Furthermore, the amount of oxygen during biofilm development should be monitored to investigate the influence of biofilm on the oxygen transportation from the air into the droplet.

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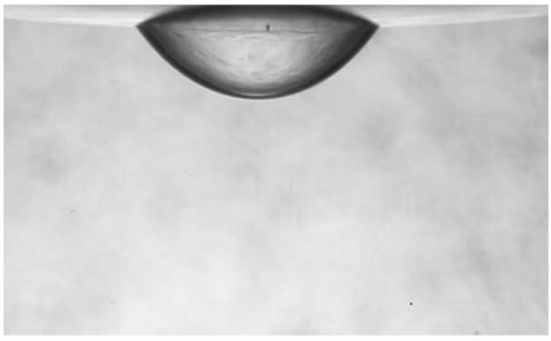
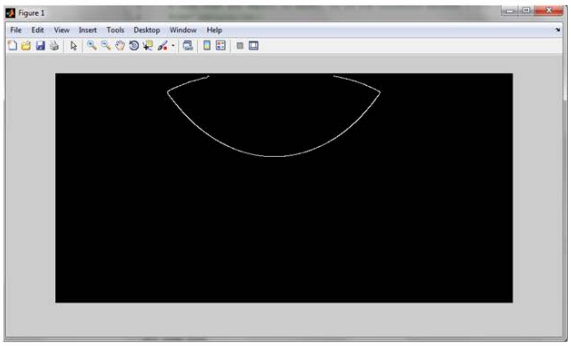
Appendix A. Modelling of water droplet

A.1 Floating water droplet containing bacteria, enzyme, SDS and NaCl on paraffin oil

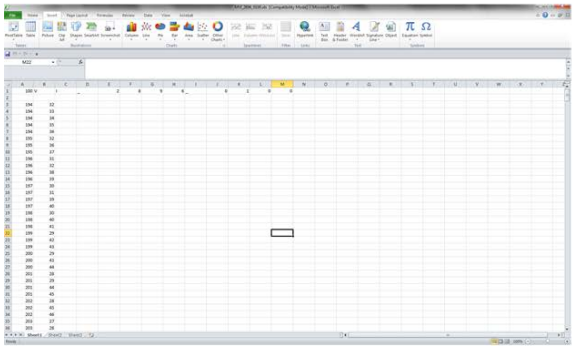
MVI_2896_100 (V = 7.24 ×10^{-5} mm^3)



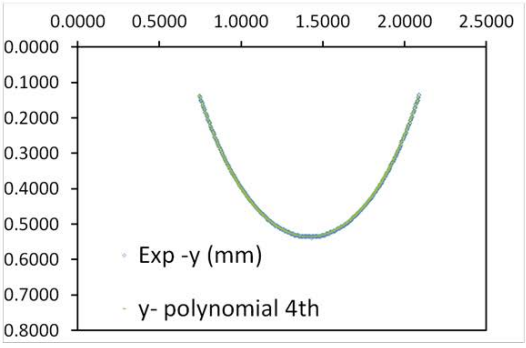
Edge fitting image using MATLAB



Excel sheet with droplet profile data

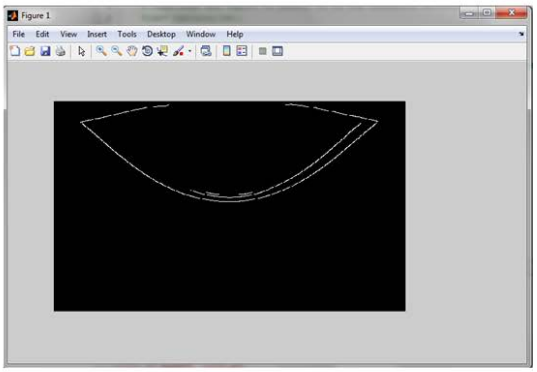


Edge fitting resulting by using a polynomial function

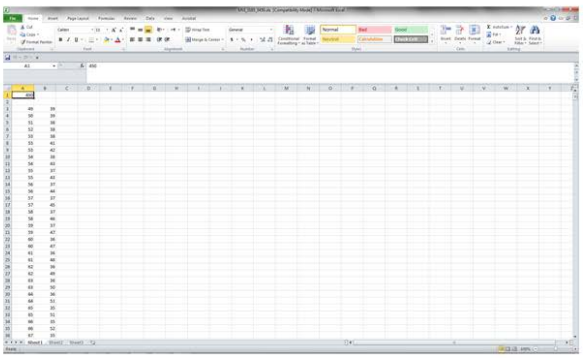


MVI_3185_450 (V = 0.798 mm^3)

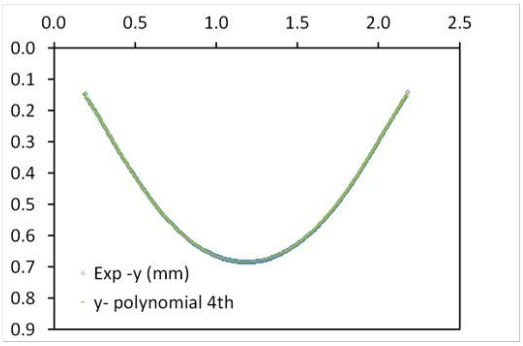
Edge fitting image using MATLAB



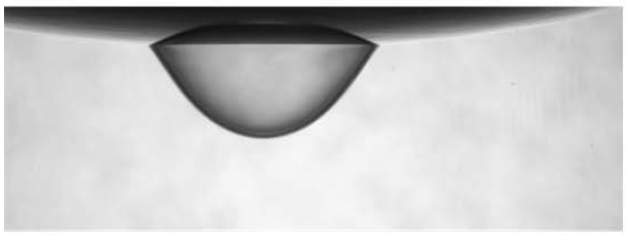
Excel sheet with droplet profile data



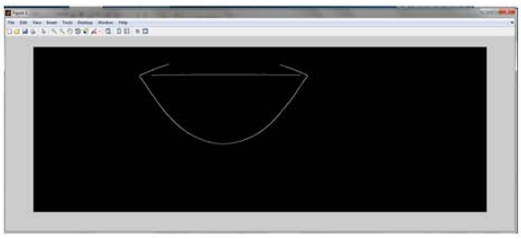
Edge fitting resulting by using a polynomial function



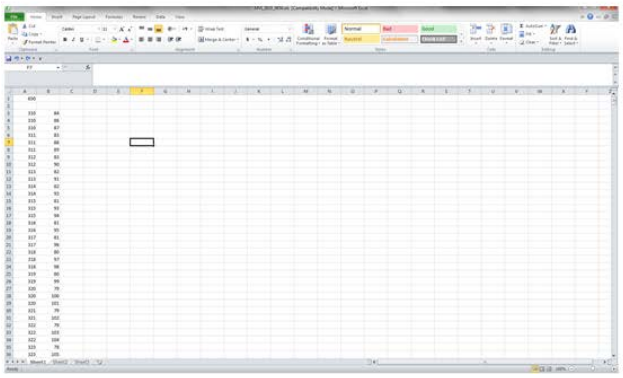
MVI_2819_650 (V = 2.39 ×10^{-3} mm^3)



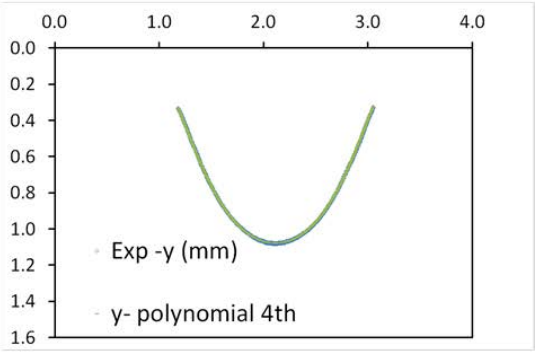
Edge fitting image using MATLAB



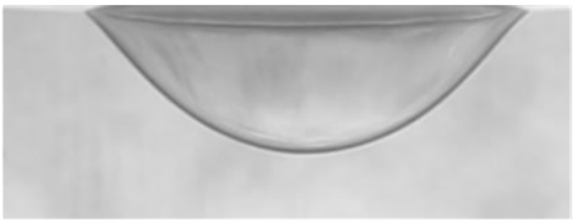
Excel sheet with droplet profile data



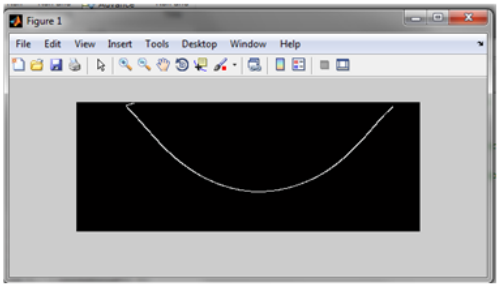
Edge fitting resulting by using a polynomial function



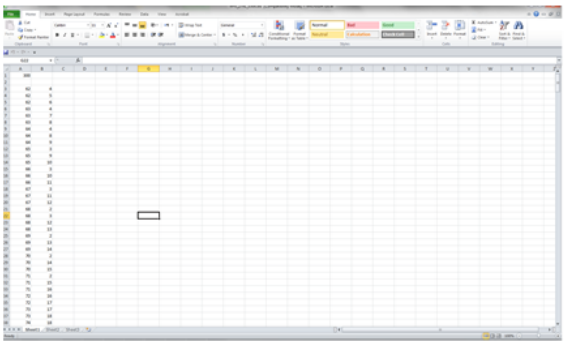
MVI_2791_300 (V = 2.52 ×10^{-5} mm^3)



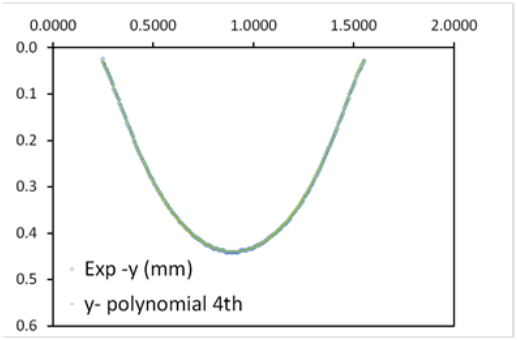
Edge fitting image using MATLAB



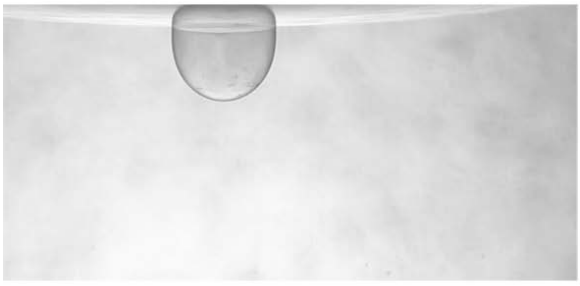
Excel sheet with droplet profile data



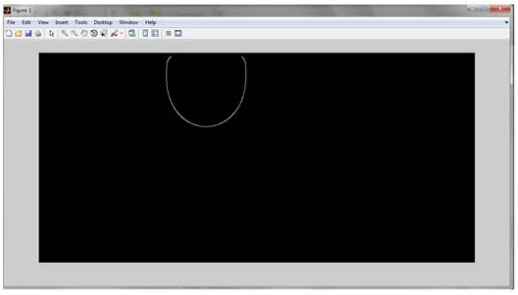
Edge fitting resulting by using a polynomial function



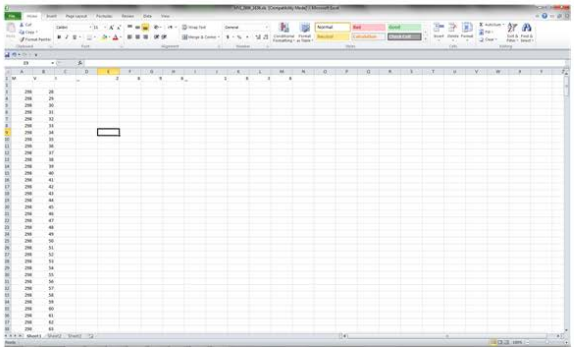
MVI_2896_1637 (V = 7 ×10^{-6} mm^3)



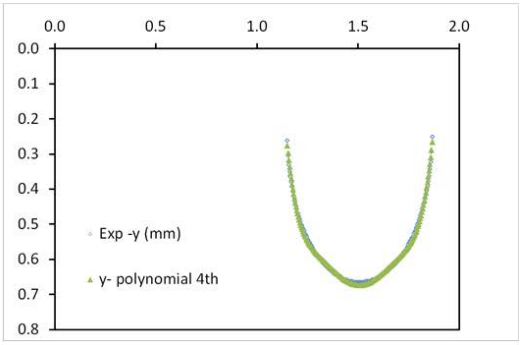
Edge fitting image using MATLAB



Excel sheet with droplet profile data



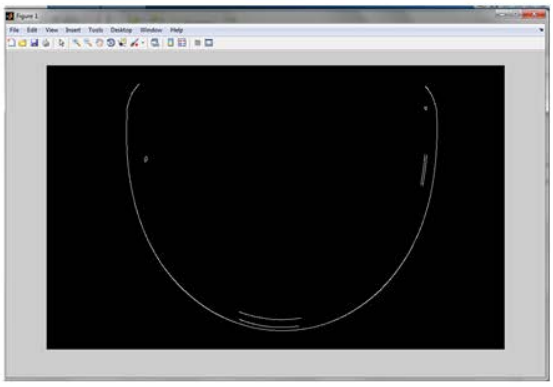
Edge fitting resulting by using a polynomial function



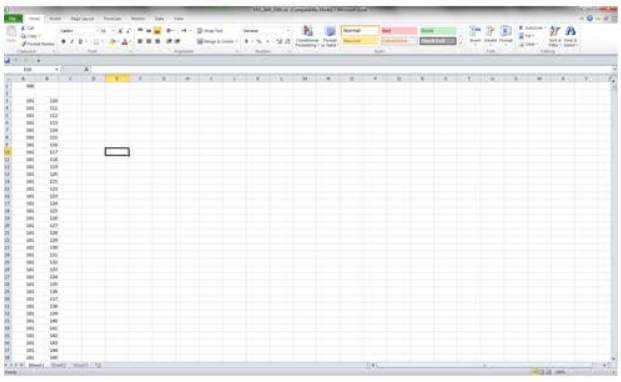
A.2 Floating water droplet containing bacteria, MLA medium, SDS and NaCl on paraffin oil

MVI_2740_360 (V = 4.28 mm^3)

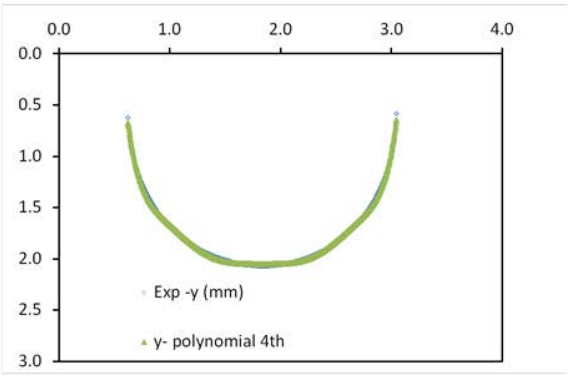
Edge fitting image using MATLAB



Excel sheet with droplet profile data

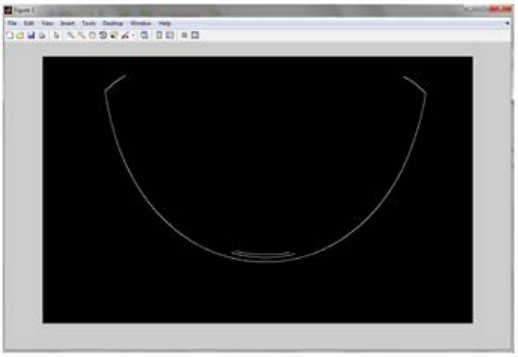


Edge fitting resulting by using Polynomial function

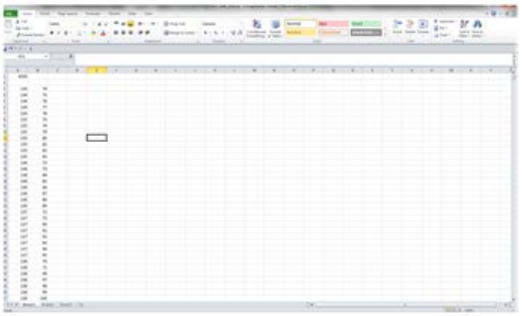


MVI_2840_4250 (V = 4.81 mm^3)

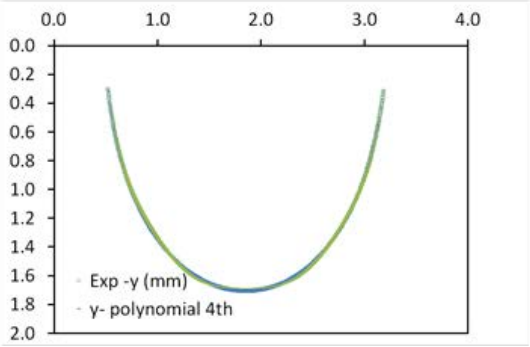
Edge fitting image using MATLAB



Excel sheet with droplet profile data

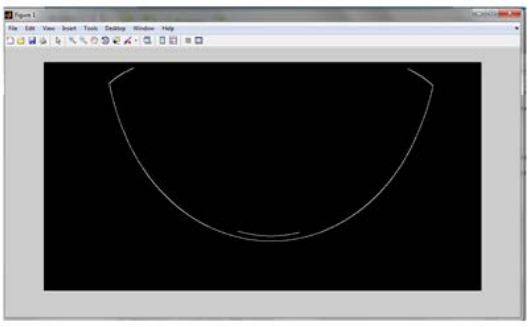


Edge fitting resulting by using Polynomial function

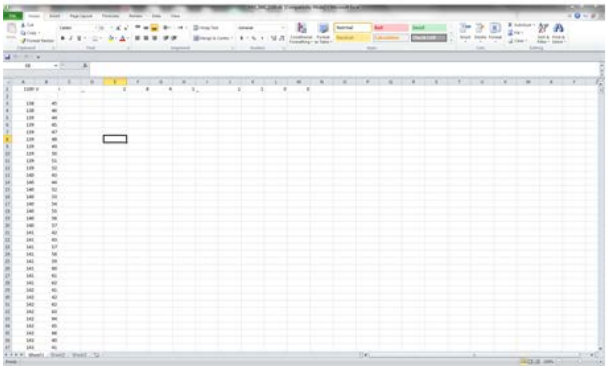


MVI_2841_1100 (V = 4.16 mm^3)

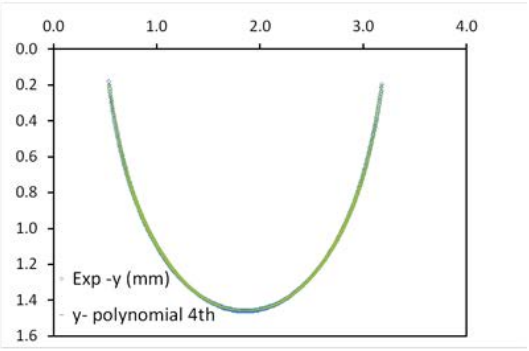
Edge fitting image using MATLAB



Excel sheet with droplet profile data

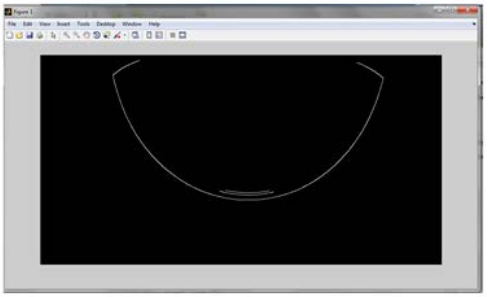


Edge fitting resulting by using Polynomial function

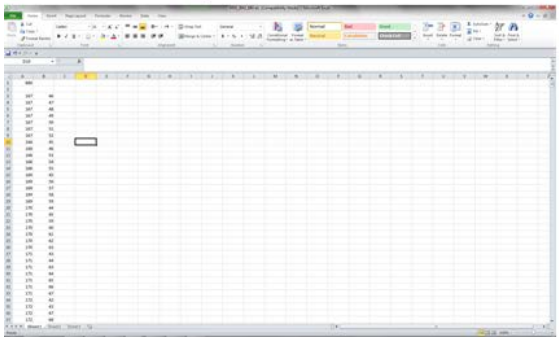


MVI_2842_880 (V = 2.92 mm^3)

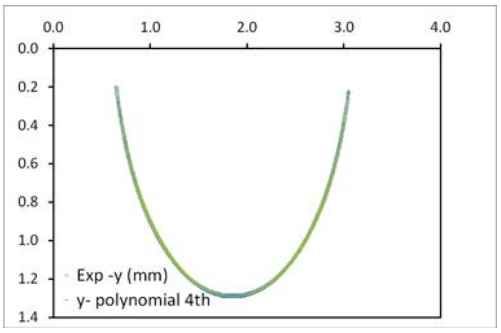
Edge fitting image using MATLAB



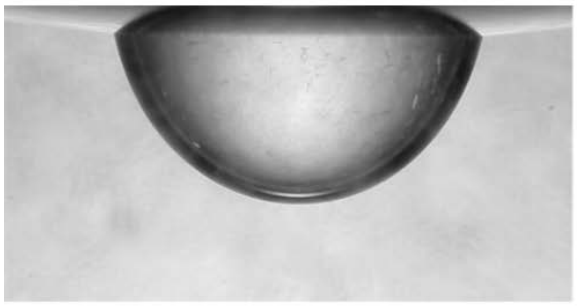
Excel sheet with droplet profile data



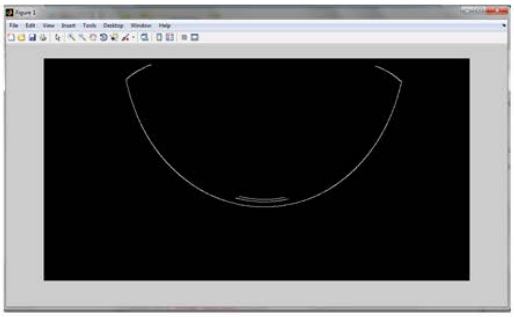
Edge fitting resulting by using Polynomial function



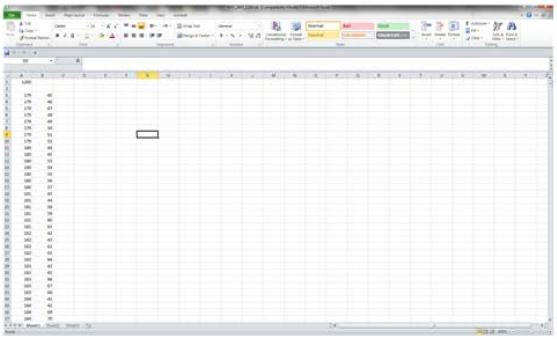
MVI_2843_1200 (V = 2.59 mm^3)



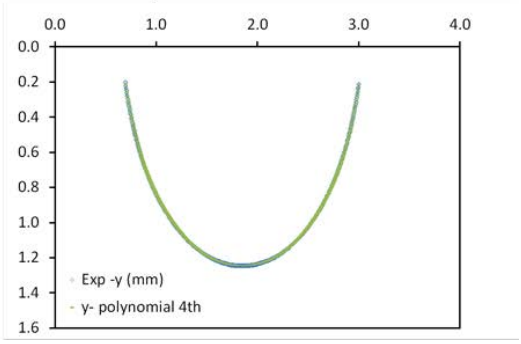
Edge fitting image using MATLAB



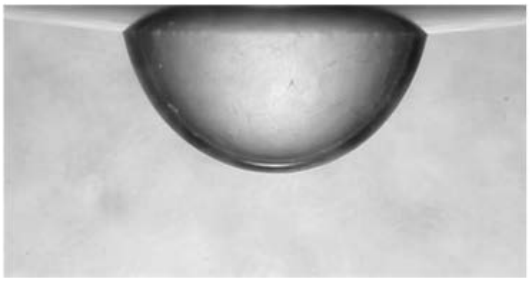
Excel sheet with droplet profile data



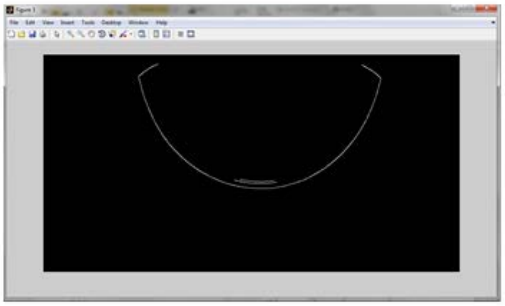
Edge fitting resulting by using Polynomial function



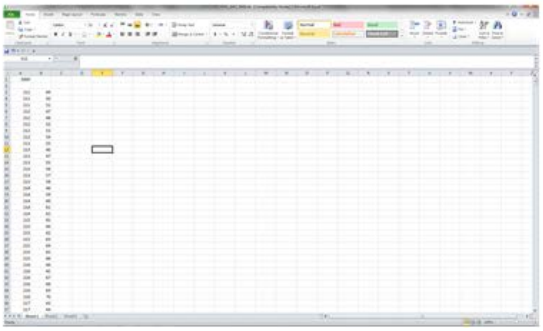
MVI_2843_5000 (V = 1.92 mm^3)



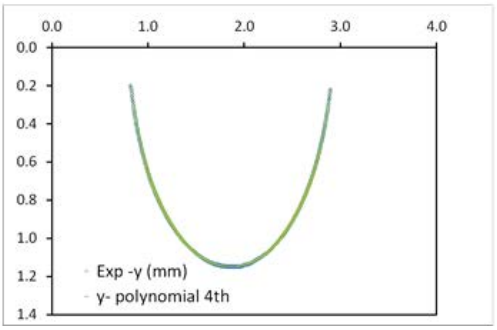
Edge fitting image using MATLAB



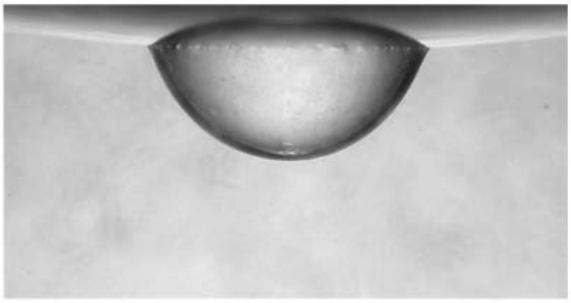
Excel sheet with droplet profile data



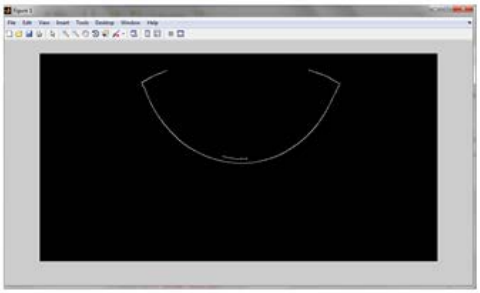
Edge fitting resulting by using Polynomial function



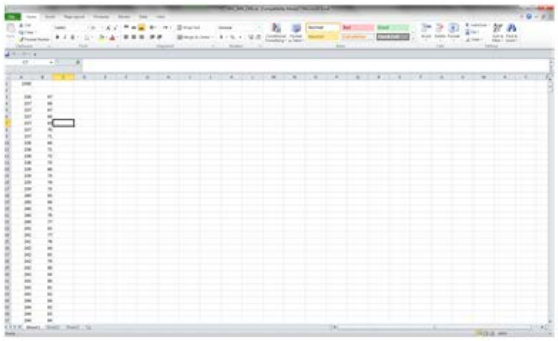
MVI_2844_1500 (V = 0.89 mm^3)



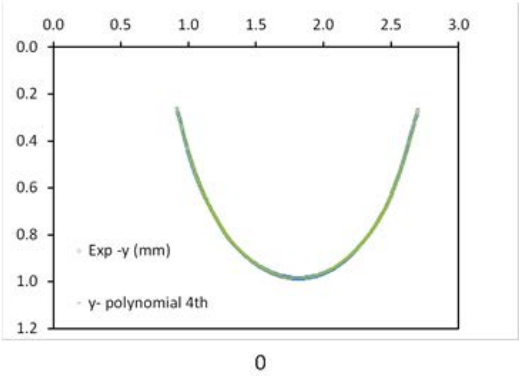
Edge fitting image using MATLAB



Excel sheet with droplet profile data

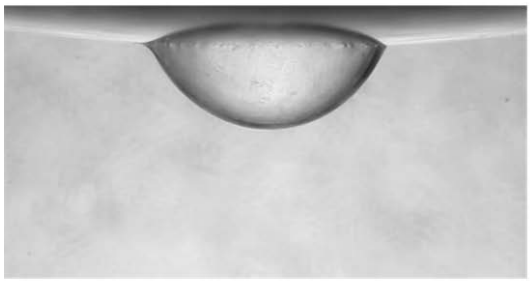


Edge fitting resulting by using Polynomial function

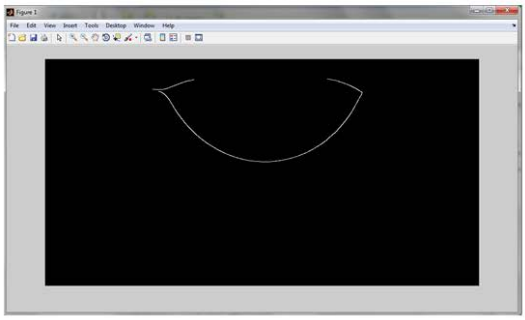


0

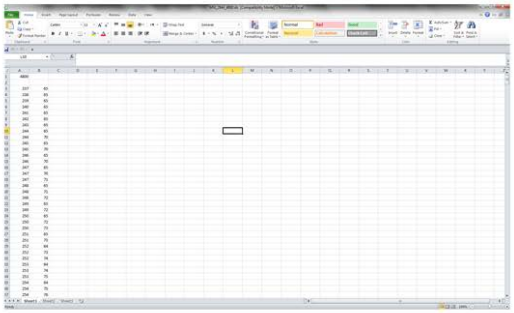
MVI_2844_4800 (V = 0.61 mm^3)



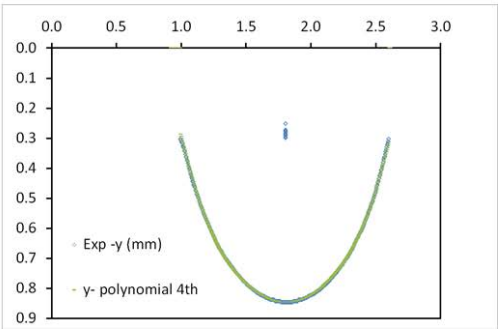
Edge fitting image using MATLAB



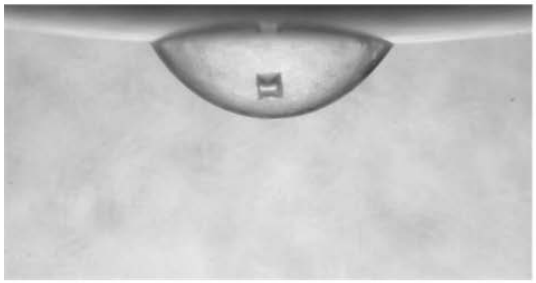
Excel sheet with droplet profile data



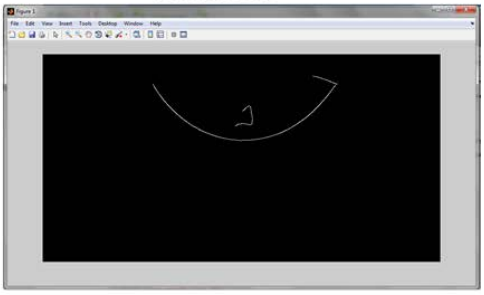
Edge fitting resulting by using Polynomial function



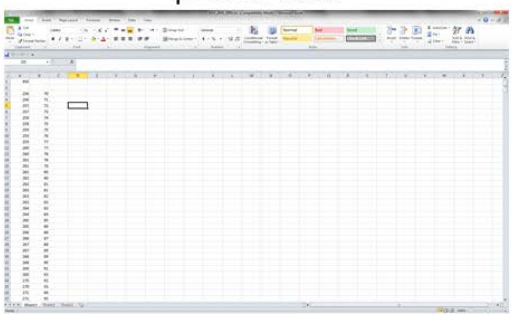
MVI_2845_950 (V = 0.56 mm^3)



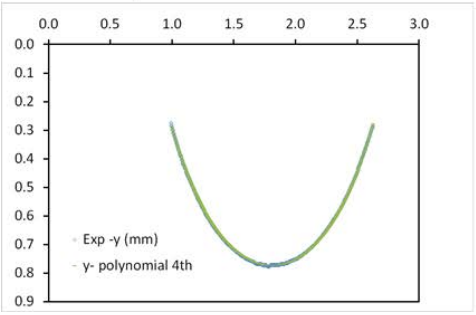
Edge fitting image using MATLAB



Excel sheet with droplet profile data



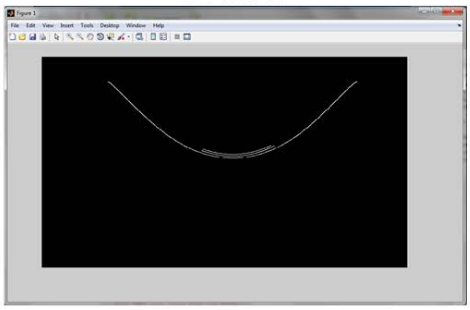
Edge fitting resulting by using Polynomial function



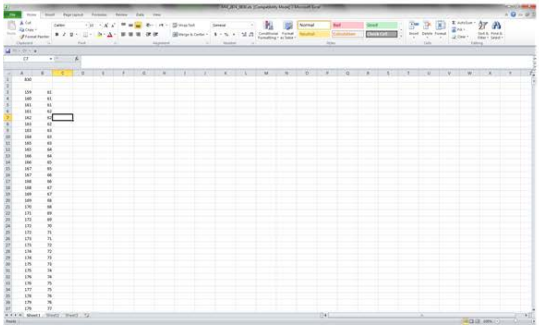
A.3 Floating water droplet containing bacteria, enzyme, Triton 705 and NaCl on paraffin oil

MVI_2874_830 (V = 1.36 mm^3)

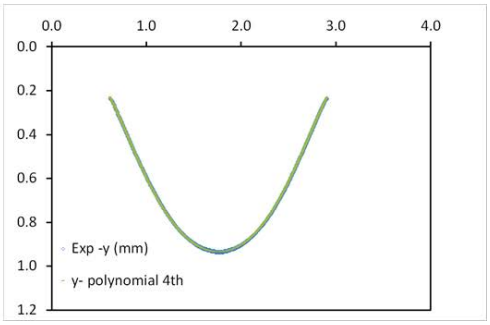
Edge fitting image using MATLAB



Excel sheet with droplet profile data

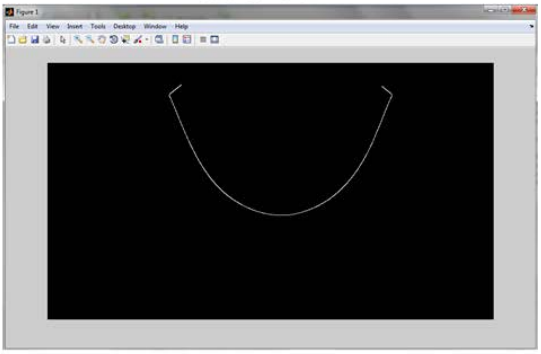


Edge fitting resulting by using Polynomial function

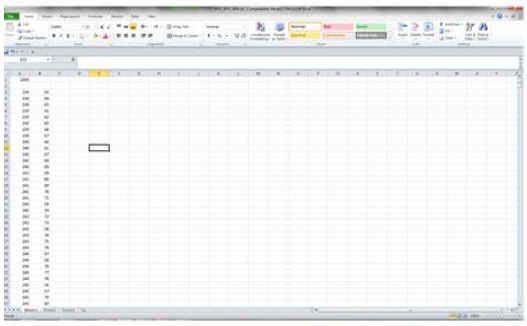


MVI_2874_1850 (V = 0.84 mm^3)

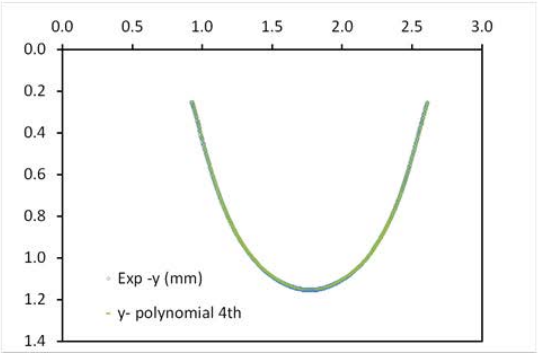
Edge fitting image using MATLAB



Excel sheet with droplet profile data

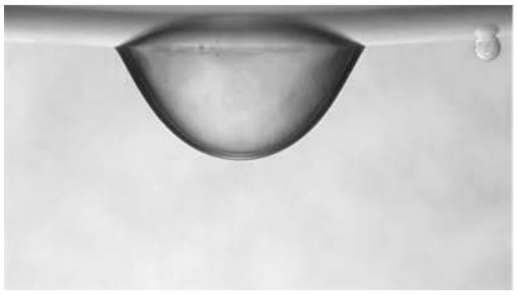


Edge fitting resulting by using Polynomial function

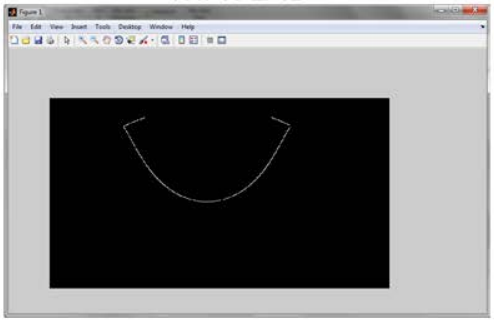


A.4 Floating water droplet containing bacteria, enzyme, SDS and NaCl on diesel oil

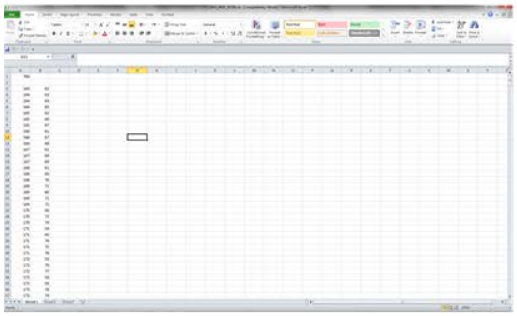
MVI_8095_700 (V = 0.43 mm^3)



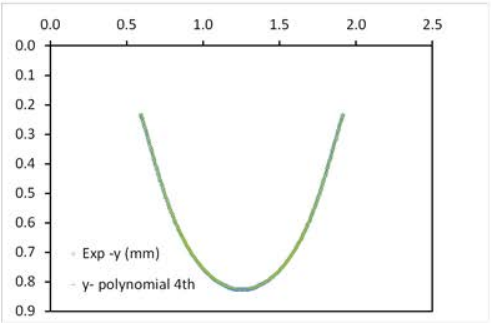
Edge fitting image using MATLAB



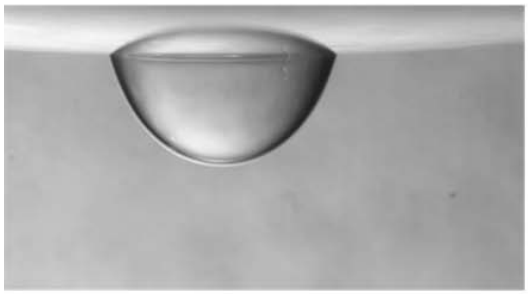
Excel sheet with droplet profile data



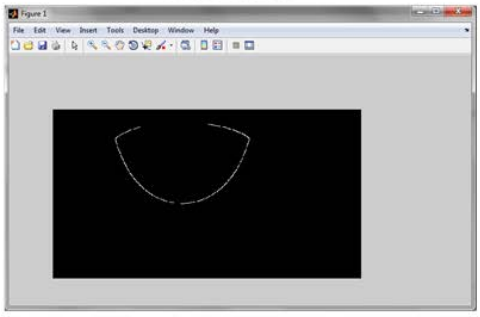
Edge fitting resulting by using Polynomial function



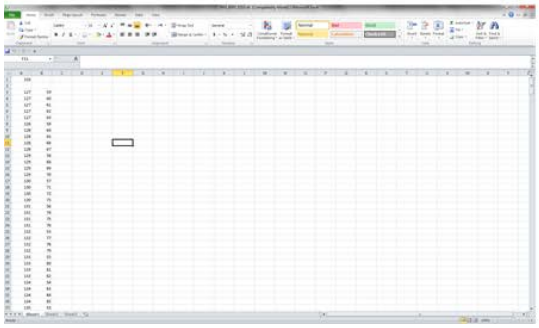
MVI_8097_310 (V = 0.198 mm^3)



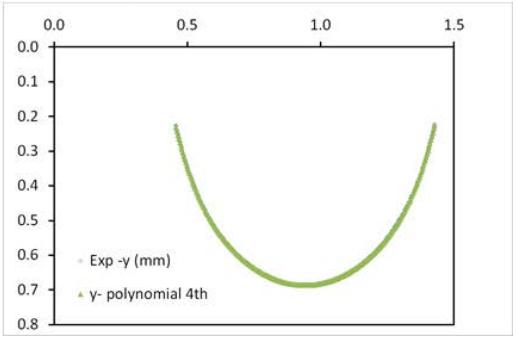
Edge fitting image using MATLAB



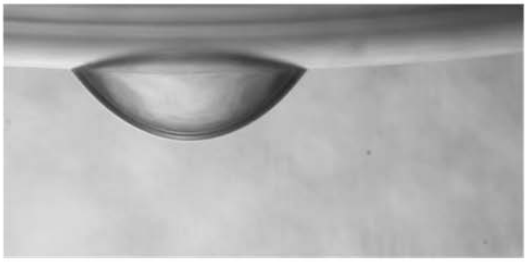
Excel sheet with droplet profile data



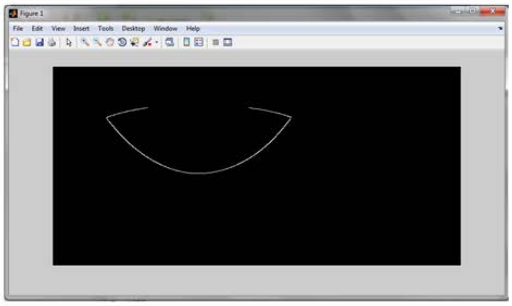
Edge fitting resulting by using Polynomial function



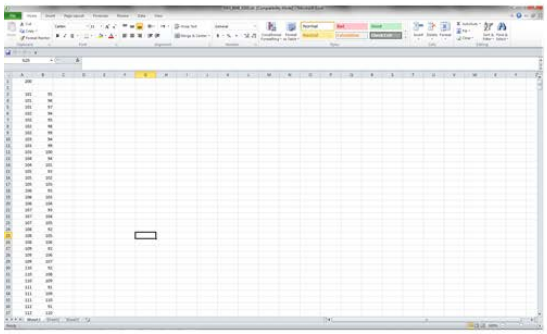
MVI_8098_200 (V = 0.23 mm^3)



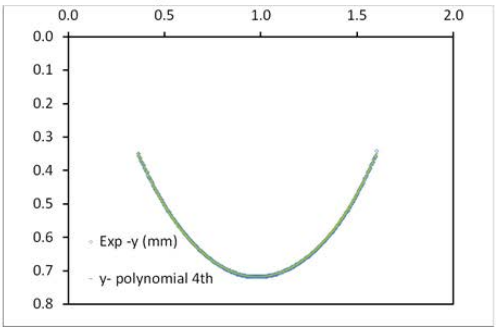
Edge fitting image using MATLAB



Excel sheet with droplet profile data

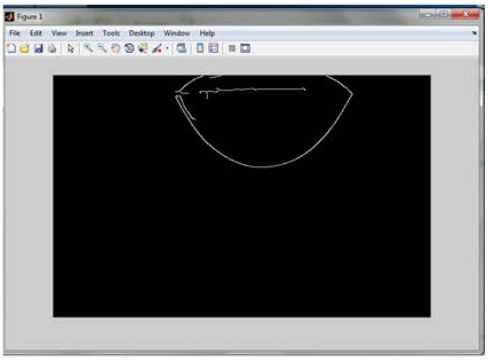


Edge fitting resulting by using Polynomial function

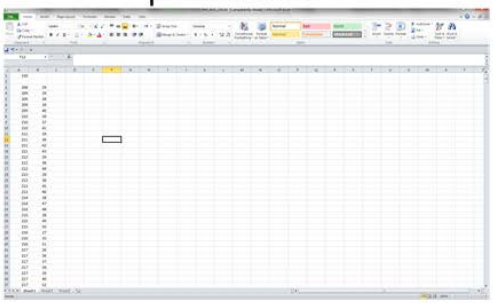


MVI_8191_150 (V = 0.24 mm^3)

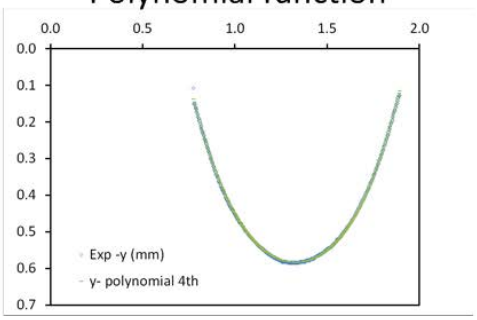
Edge fitting image using MATLAB



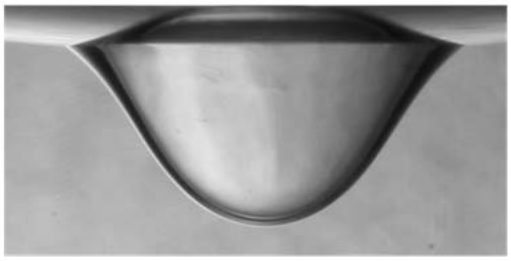
Excel sheet with droplet profile data



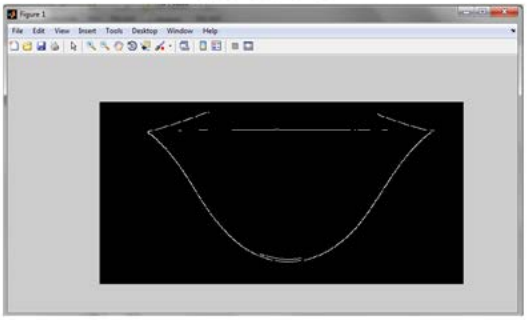
Edge fitting resulting by using Polynomial function



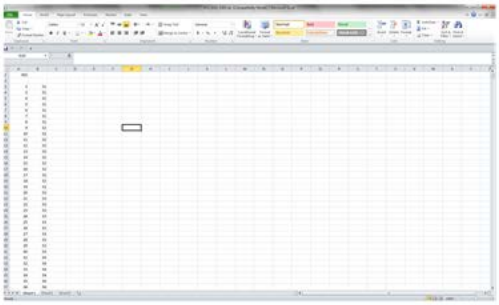
MVI_8192_302 (V = 1.32 mm^3)



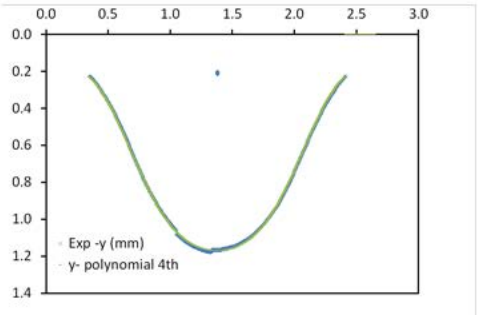
Edge fitting image using MATLAB



Excel sheet with droplet profile data



Edge fitting resulting by using Polynomial function



Appendix B MATLAB edge detection code

```
numlength =4;  % Length of numbers in filename including padded zeros
basefilename = 'MVI_8100_';
fmt = ['%0' num2str(numlength) 'u'];    % Zero padded integer of specified
length

for i =0424:0425
  k= 0001+ i ;
filename = [basefilename num2str(k,fmt)];
disp(filename)
im=imread( filename, 'jpg' );
threshold=0.5;
im= rgb2gray(im);
Matrix = edge(im, 'canny', threshold);
imshow(Matrix);
[row,col] = find(Matrix);
xlswrite (filename, k ,1, 'A1');
xlswrite (filename, col,1, 'A3');
xlswrite (filename, row,1, 'B3');

end
```